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THE INFLUENCE OF WINTER STEM PRUNING ON SUBSEQUENT STEM- AND ROOT-DEVELOPMENT IN THE APPLE

By R. C. KNIGHT

Research Institute of Plant Physiology, Imperial College of Science and Technology, and East Malling Research Station

In the course of any study of the influence of rootstock upon scion it is obviously necessary that cognizance be taken of the reciprocal influence of scion upon rootstock. It is important, for example, to know how far the well-defined characters which are associated with different varieties of rootstock may be influenced by the nature and condition of the scions.

Concurrently, therefore, with the study of rootstock influence, experiments have been carried out on the effect upon the root system of different scions and of scions under different growth conditions.

One way in which scion growth can be profoundly affected is, of course, by stem pruning and from time to time reports have been issued recording the the effect of different types and degrees of stem pruning on scion performance. The study of the response of the root system to stem pruning has lagged somewhat behind that of scion performance, but a progress report appeared in 1924 (5, pp. 113-15) indicating the method of experiment which was being followed and the general trend of the results so far obtained. This report foreshadowed the extension of the experiment, and since its publication the subject has been investigated further, applying a more elaborate technique to a fairly wide range of material. The investigation has been confined almost entirely to winter pruning, although a few trials of summer pruning have given interesting results, similar to those indicated in the earlier report referred to above. The significance of the results of summer pruning is still obscure and it is not proposed here to discuss them further. The present report deals only with the influence of different degrees of winter stem pruning upon subsequent shoot- and root-growth.

The varieties used were Bramley's Seedling, Cox's Orange Pippin, Grenadier, Lord Derby, Newton Wonder and Stirling Castle, whilst the rootstocks were Broadleaf (No. I), Doucin (No. II), Jaune de Metz (No. IX) and Nonesuch (No. VI), a selection which comprised a wide range of root character. Including the unworked stocks used as controls and a series of unworked stocks of the unnamed variety No. X, the number of series treated amounted to twenty-one, comprising over 500 trees. Measurements were made and weights taken of both stem- and root-growth of each tree before planting and each series was divided into a number of batches, so that not only was the average size of the trees in each batch almost exactly the same, prior to the pruning treatment, but also each batch exhibited a similar range of vigour. This method of selecting material falls far short of the ideal and results in a considerable variation of size of tree within a batch, but there are, of course, practical difficulties in the way of raising a sufficiently large number of trees to permit the selection of the required number of individuals having exactly the same vigour. The number of batches into which a series was divided varied from two to seven, each being subjected to a different pruning treatment. The number of individuals in a batch ranged from five to fourteen and it is realized that these numbers would be inadequate from which to draw conclusions if only one or two experiments were carried out. Since, however, the investigation comprises twenty-one separate experiments using different combinations of stocks and scions, it is considered that the uniformity of the results renders them of real significance, even though the unit of trees was often small.

In the previous experiments on the subject (5) records were taken of the amount of new shoot- and root-growth produced in the season following planting and pruning. In order to determine the amount of roots developed in the season, all older roots were removed from the trees prior to planting. It may be noted that whilst it is a simple matter to detect the junction between shoots of any one year and those of the previous year, it is quite impossible by inspection to assign roots to their year of formation. Even the criterion of xylem rings is quite unsatisfactory because the number of more or less well-defined rings in a root is usually greater than the number of years since its first emergence. Therefore, unsatisfactory though it may be in some respects, it appears to be essential to remove all roots prior to planting if the amount of root-growth during the following season is to be determined. Fortunately, when roots have been removed from a stem in this manner, new roots very readily develop when the tree is replanted (5, pp. 115-17), and although some varieties of rootstock appear to suffer more than others from the treatment if a dry season follows, the growth made is generally satisfactory.

After measurements and weights had been recorded, therefore, the trees for the present experiments, deprived of their roots, were planted in rows, special care being taken to pack the soil thoroughly round their bases. In addition, artificial support was found to be necessary at the outset to prevent loosening, which is so liable to occur in the absence of an anchoring root system.

Pruning was done immediately after planting and some combination of two or more of the following treatments was applied to each series:

Batch.	*Amount of stem removed.*
A.	Nil.
B.	One half of length of one year old wood.
C.	Two thirds ,, ,, ,, ,,
D.	Whole ,, ,, ,, ,,
E.	One third of length of two year old wood.
F.	One half ,, ,, ,, ,,
G.	Two thirds ,, ,, ,, ,,
H.	Whole ,, ,, ,, ,,

Pruning the whole of the two year old stems involved in some cases the removal of some fifty per cent. of the total weight of the tree.

The plants were allowed to grow for a season and were then carefully lifted and washed. The new roots were severed as close as possible to the stem and were graded according to diameter. The new shoots produced during the year were then removed and weighed and the remainder of the plant, which consisted of the original unit after pruning together with new tissues laid down on the old framework during a year's growth, was also weighed. Thus it was possible to obtain by difference the increase in weight of the old stems. In the experiments reported earlier, no account was taken of this increase, only new shoots being recorded. All weights taken were those of the material in the fresh condition, since it was impracticable to obtain final dry weights of the large number of trees used, and in any case, only fresh weights could be obtained of the trees prior to planting.

As is usual in such experiments, the various operations involved in the preparation, planting, pruning and recording of the trees were possible only with the co-operation of several members of the propagation and recording staffs, and indeed many of the trees used were actually raised for the purpose at the instigation of Messrs. R. G. Hatton and J. Amos, whilst Mr. R. G. Hatton, Mr. J. Amos and Mr. N. H. Grubb have contributed welcome and helpful criticism throughout. Grateful acknowledgment is made of this indispensable assistance.

PRUNING AND STEM GROWTH.

It is, of course, well known that one of the effects of pruning is to increase the vigour of the new shoots which are subsequently developed and this was shown quantitatively by the results obtained in the earlier series of experiments (5, p. 114) and in that case, the severe pruning of Series C. resulted in a smaller increase over the control than did the less severe pruning. An exactly similar result was obtained in the present experiments. Two series of unworked stocks were divided into six and seven batches respectively and each batch was subjected to a different degree of pruning. The resulting new shoot-growth of one of these series is shown in Table I.

TABLE I.

Influence of Pruning on subsequent New Shoot-Growth.

Unworked Apple Stock Broadleaf (*No.* 1) *four years old.*

Degree of Pruning.	% of Tree Removed in Pruning.	Weight of Subsequent New Shoots.
Nil	0	13·3
Two-thirds of 1 year old stem	5	15·1
Whole ,, ,, ,, ...	11	22·4
One-third of 2 year old stem	22	25·7
Two-thirds ,, ,, ,, ...	33	30·3
Whole ,, ,, ,, ...	50	20·2

Here the weight of new shoots produced increased steadily with the severity of pruning until the latter reached a stage when thirty-three per cent. of the tree was cut away, beyond which (in this case as also in a series of unworked stocks, No. X) the weight of new shoots produced was relatively low.

Whilst the development of new shoots is a prominent and easily measured feature of growth, it does not represent the whole of the increase of stem weight, and is not necessarily the greatest part of this increase, nor even proportional to it. With the development of buds into shoots new tissues are laid down in older stems, which show a corresponding increase in girth and weight. Thus the total *new stem-growth* is made up of *new shoot-growth,* consisting of newly developed organs, together with the increase in size of older stems, which may for brevity be termed "*secondary stem-growth*". In the present experiments the technique already described rendered possible the determination of the weight of secondary stem-growth as well as new shoot-growth. In addition, measurements of girth at the beginning and the end of the season gave a further indication of the thickening of the main stem.

Table II shows the stem-growth made by a sample of the twenty-one experiments, each figure representing the average of a batch of from five to fourteen trees. Columns 4, 5 and 6 show results similar to those in Table I, over a smaller range of severity of pruning, but a totally different tendency is observed when "secondary stem-growth" is considered (Columns 6, 7 and 8). Here the effect of pruning is the reverse of that upon new shoot production, the season's increment in weight of the older stems decreasing with increased severity of pruning. It must be remembered, however, in interpreting this decrease that the removal, during pruning, of portions of the previous year's stems automatically reduces the framework upon which the new tissues are to be laid down. Thus a decrease in the amount of new thickening does not

TABLE II.

Weight of Stem Produced in Season following Pruning.

Variety.	Stock.	Age. Years.	New Shoots.			Thickening of Older Stems.			Total.		
			Pruning Treatment.								
			A	C	F	A	C	F	A	C	F
Cox -	IX	2	4·8	7·0	8·5	23·5	8·5	12·3	28·3	15·5	20·8
,, ...	I	2	5·2	10·9	11·9	25·8	15·2	8·3	31·0	26·1	20·2
Bramley ...	I	2	10·4	11·1	11·9	37·6	23·3	20·4	48·0	34·4	32·3
(Unworked) ..	I	2	12·0	17·1	21·9	75·6	41·4	25·0	87·6	58·5	46·9
Bramley ..	I	1	11·3	14·6	16·7	53·1	30·8	25·1	64·4	45·4	41·8
Derby	VI	1	11·1	10·1	—	53·7	17·9	—	64·8	28·0	—
Newton ..	VI	2	—	7·5	7·9	—	22·6	11·9	—	30·1	19·8

necessarily indicate less intense or less prolonged activity of the cambium. A study of the amount of new thickening in relation to the weight of old stem remaining after pruning might be expected to give a more accurate representation of the influence of pruning upon secondary stem-growth, but examination of the ratio reveals a lack of uniformity in the response. In about two-thirds of the possible comparisons, the ratio of new thickening to the original stem weight after pruning decreases with increased severity of pruning, whilst in the remaining cases the opposite effect was realized, so that it is impossible to reach a definite conclusion on this point.

Indications have been obtained of the existence of another factor influencing the response of both stem- and root-growth to pruning. A number of unworked stocks of the variety No. X were subjected to different degrees of pruning and the results as a whole were similar to those just described. The details of the figures for new shoot-growth, however, merit some further examination, and are shown in Table III.

TABLE III.

New Shoot Production in Relation to Pruning.

Apple Stock No. X.

Series.	Degree of Pruning.	Percentage Weight of Tree Removed.	Subsequent Weight of New Shoots.
A	Nil	0	85
B	One third of 1 year old wood	2·3	84
C	Two thirds ,, ,, ,,	7·2	103
D	Whole ,, ,, ,,	12·5	70
F	Half of 2 year old wood	22·1	102
G	Two thirds ,, ,,	35·1	116
H	Whole ,, ,,	49·5	59

The removal of two per cent. of the total weight of the tree had a negligible effect on new stem production but thereafter more severe pruning resulted in increased shoot production up to the stage when the whole of the one year old wood was removed, following which a marked decrease in new stem occurred. Beyond this stage further increases were recorded up to the series in which the plants were pruned to ground level.

Whilst the buds which produced the new shoots on the trees in Series A, B and C (Table III) were normal buds laid down the previous season, the conditions were altered in the following series. The removal of all the one year old wood in Series D necessitated the development of leading shoots from buds on the two year old wood, which would normally have remained dormant, or which may have been adventitious, and the low weight of the resulting new shoots may be associated with this fact. The decrease in Series H similarly may be ascribed to the development of shoots from buds at or below ground level.

This result indicates that the figures of new shoot-growth in Tables I and II may be an incomplete expression of the facts and it is possible that the smooth curves obtained in those cases resulted from the omission to include degrees of pruning which as Table III indicates, produce particular effects.

However, the general result has been confirmed in all these experiments that pruning decreases total new stem-growth and at the same time profoundly alters its distribution. The two most extensive experiments will show this change of distribution if new shoot-growth and secondary growth are expressed as percentages of the total stem-growth as in Table IV.

TABLE IV.

Distribution of Total Stem-Growth in Relation to Pruning.

Stock No. X.			Stock No. I.		
Percentage of Tree removed in Pruning.	New Shoots.	Thickening of old stems.	Percentage of Tree removed in Pruning.	New Shoots.	Thickening of old stems.
	Per cent.				
0	15	85	0	15	85
2·3	21	79			
7·2	31	69	5·0	29	71
12·5	37	63	11·4	37	63
22·1	52	48	21·6	38	62
35·1	62	38	33·2	44	56
49·5	71	29	50·4	60	40

Although differences of magnitude of response occur in the two experiments, as indeed might be expected with different varieties, the figures indicate the large changes of distribution of stem-growth produced by different degrees of pruning. In the unpruned tree some eighty-five per cent. of the total "stem effort" takes the form of thickening on old stems, whilst when half of the plant is removed in pruning this proportion is reduced to some thirty or forty per cent. Exactly similar results were obtained with the nineteen other series in which, however, the smaller numbers available permitted the use of only two or three pruning treatments.

PRUNING AND ROOT-GROWTH.

In the earlier report it was shown that pruning reduced subsequent root-growth and the present experiments have wholly confirmed this finding. Table V shows the figures of root production for the same trees as those in Table II.

TABLE V.

Weight of Roots Produced during Season following Pruning.

Variety.	Stock.	Age. Years.	Roots. <1 mm. Diam.			Roots. >1 mm. Diam.			Total Roots.		
			Pruning Treatment.								
			A	C	F	A	C	F	A	C	F
Cox's Orange Pippin ..	IX	2	4·1	2·7	4·0	4·5	1·6	2·8	8·6	4·3	6·8
,, ..	I	2	5·7	4·7	4·3	4·5	3·4	2·6	10·2	8·1	6·9
Bramley's Seedling ..	I	2	12·5	9·5	8·9	8·1	6·0	6·1	20·6	15·5	15·0
(Unworked)	I	2	12·6	10·2	9·4	13·4	8·8	7·7	26·0	19·0	17·1
Bramley's Seedling ..	I	1	11·2	9·2	8·5	13·6	8·7	8·1	24·8	17·9	16·6
Lord Derby	VI	1	12·0	9·3	—	11·1	4·6	—	23·1	13·9	—
Newton Wonder ..	VI	2	—	14·4	11·4	—	9·5	3·6	—	23·9	15·0

The general result is a decrease in the production of both fine and coarse roots as a result of pruning and the more severe the pruning the smaller is the weight of roots subsequently produced. In Table V the series Cox on IX does not conform to this general behaviour, but since Cox on I (and also on II) responded similarly to other series, the unusual result cannot be regarded as a varietal phenomenon. Of some sixty different batches treated only five gave results not conforming to the general rule. It may be that the aberrant cases are the result of using small number of trees in some units and do not represent a real difference in response, but on the other hand it is possible that a finer analysis would show that the graph of weight of roots produced does not fall steadily with increased severity of pruning, showing instead some secondary maxima. This point has already been discussed in relation to shoot-growth.

The efficiency of a root system as an absorbing agent depends partly on the area of potential absorbing surface. It is, of course, impracticable with large numbers of trees to excavate the root systems so that the young root extremities which carry the absorbing areas are obtained entire, and it would be equally difficult to make a quantitative estimation of the absorbing area if it were excavated. Behind the absorbing region the root increases in diameter up to its point of insertion on the stem or other root, so that if roots are graded according to their diameter the number of fine rootlets may be regarded as being roughly proportional to the number of absorbing tips originally present. The weight of so-called "fibrous" roots has therefore been recorded as an indication of the extent of the absorbing capacity of the system. It is well known that different varieties of apple root systems have different proportions of fibre and coarse root but it is not known how far this proportion is altered by such treatment as branch pruning.

Whilst the figures in Table V show the quantitative effect of pruning on root production, it is also desirable to know whether pruning changes the character of the root system by altering the proportion of fibre which is present. The percentage of fibre present has sometimes been taken as a characteristic expression of a root system, but evidence is now available showing that such a percentage must be considered in relation to the size of the whole root system if it is to be regarded as a reliable indicator of root character. This question has been dealt with elsewhere (1) and a full report of later observations is in the press (6).

So far as they go, however, the figures of the proportion of "fibre" (in these experiments, all roots below 1 mm. diameter were grouped in this class) in the root systems are instructive. The influence of different scion varieties on the proportion of fibre in any one variety of root system has already been discussed elsewhere (1, 4, 6), but some further evidence is available here together with the figures obtained after pruning. The question is, of course,

TABLE VI.

Proportion of "Fibre" in No. I Root System as influenced by Pruning and Scion Variety.

Scion Variety.	Treatment.	Total Weight of Roots.	% Fibre.
Cox	A	10·2	56
,,	B	8·1	57
,,	C	6·9	62
Bramley	A	20·5	61
,,	B	15·5	61
,,	C	15·0	59
(Unworked)	A	26·1	48
,,	B	18·9	54
,,	C	17·0	55

complicated by the difference in size of root system resulting from different degrees of pruning, which in itself may influence the figures for "percentage fibre", but Table IV shows that apart from the usual tendency of fibre to decrease with increasing weight of total roots, the influence not only of scion variety but also of different degrees of pruning upon the proportion of fibre is very small.

These figures alone are, of course, quite inadequate to allow definite conclusions, in view of the small numbers of trees used, but similar results were obtained in all series, and the only case showing considerable divergence from the series was that of Grenadier one year old, which, as has been recorded before (1), showed a low proportion of fibre on No. VI.

THE SHOOT/ROOT RATIO.

Whilst pruning is followed by marked response in the weight of new roots and new stem, and also by the re-distribution of the total new stem-growth, a different picture is presented when the relation of new stem-growth to new root-growth is considered. In every experiment of the whole series it has been found impossible to alter this relationship to any considerable extent by any pruning treatment applied. The figures are presented in Table VII by expressing total new stem-growth (new shoots and thickening of old stems) as a percentage of the total growth (new shoots + thickening of old stems + new roots).

TABLE VII.

Influence of Pruning on the percentage of Total Growth appearing as Stem-Growth.

Variety.	Stock.	Age. Years.	New Stem-Growth as Percentage of Total New Growth.		
			Unpruned.	Two-thirds one year wood.	Half two year wood.
Cox	IX	2	77	78	77
Bramley	IX	2	74	73	70
(Unworked) ..	IX	2	78	73	76
Cox	II	2	77	80	—
Bramley	II	2	84	80	83
(Unworked) ..	II	2	86	82	82
Cox	I	2	75	76	75
Bramley	I	2	70	69	68
(Unworked) ..	I	2	77	76	74
Grenadier ..	VI	1	68	65	—
Grenadier ..	VI	2	61	59	—
Lord Derby ..	VI	1	74	67	—
Lord Derby ..	VI	2	64	—	69
Newton Wonder	VI	1	72	68	—

The largest difference resulting from different degrees of pruning is seven per cent. (Lord Derby on No. VI, one year old) and the figures throughout are remarkably constant for any one variety, stock and age. Similar uniformity was obtained in the two larger series referred to in Table IV, the percentages for the seven batches of Stock No. X, 1924, being 81, 82, 79, 79, 80, 81 and 80 whilst those for the six batches of No. I, 1927, were 77, 75, 74, 75, 75 and 78, the unpruned batch being quoted first in each case.

Whilst the ratio of new shoot to new root is remarkably uniform in any one series of trees in spite of widely different pruning treatments, it appears that different ratios may be obtained with different varieties, and Grenadier is an outstanding example of this, having a relatively high proportion of root. This characteristic of Grenadier has been noted frequently on various occasions (1, 5).

It is probable also that the figures will differ with different rootstocks and with trees of different ages, though the evidence on this question is incomplete.

Whilst the ratio of new stem-growth to new root-growth is constant, and independent of pruning treatment, that ratio of total stem (including that remaining after pruning) to new root is far from uniform. One of the factors contributing to this is the difference in the rate of recovery of the trees from the severe treatment, prior to planting, necessitated by the complete series of records required. Some trees started growth later than others and thus the total amount of new growth made was not proportional to the weight of the tree after pruning so that the ratio of total stem (old and new) to total root (wholly new) was very variable.

The uniformity of the ratio of new stem to new root may be regarded as a reflection of the close inter-dependence of the two types of growth. The carbohydrates for root formation are supplied by the leaves, and mineral elements used in stem-growth are provided by the absorption processes of the new roots; the amount of stem formed being thus regulated by the absorptive capacity, and therefore presumably the size, of the root system, and vice versa. The explanation, however, is probably not quite so simple as this. It is not certain that in altering the amount and distribution of stem-growth, leaf area is altered proportionally. On short spurs, leaves are crowded together, whilst on leaders and strong laterals they are widely spaced and pruning alters the relative amount of new shoot-growth which takes these two forms, as well as altering the proportion of new stem-growth appearing as new shoots.

Evidence is lacking as to the effect of pruning on the relation of leaf area to new stem-growth, but Austin has shown (2) that in the apricot, whilst hard pruned trees have a smaller leaf area earlier in the season than lightly pruned ones, the former may make up the deficiency before the end of the year and may actually surpass the lightly pruned trees. In attempting to estimate the assimilating capacity of pruned and unpruned trees, it would be necessary, therefore, to take cognizance of such factors as time and illumination as well as leaf area, but this is at present impracticable.

The results contribute some information relevant to transplanting practice, although certain reservations are necessary. The pruning of maiden trees on transplanting from the nursery is largely dictated by the subsequent form into which they are intended to develop, but even here the effect of pruning on total growth as well as on form is not unimportant. Oskamp (7) has summarized the results of pruning at planting time and reports results similar to those obtained in the present experiments. Heavy pruning resulted in a lower total weight and greater terminal growth, an effect which is prolonged into the second season, but Oskamp stresses the necessity of adapting pruning to the requirements of the framework of the tree.

In the case of transplanting older trees, however, where the problem appears to be less simple and the question of amount and distribution of growth is of immediate import, there exist considerable differences of opinion and practice.

Whilst the reaction of a tree to stem pruning at the time of transplanting must obviously be related to such factors as variety, stock, weather conditions and the type of growth being made by the tree prior to lifting, there appear to be two points of view in looking at the problem. First it has been the argument of one school that since in the process of transplanting there must inevitably be some damage to the roots, therefore it is necessary to prune the head of the tree to restore the balance of shoot to root. On the other hand, it is urged that if a tree is headed back, the new growth in the first season is much less than that produced by a similarly pruned tree which has not been disturbed, and is not very much more than that produced by the unpruned tree. If, however, pruning is delayed until a year after transplanting, the growth made in the second year is much greater than that of a tree pruned at transplanting. In fact by the end of the second season, the tree not pruned till a year after planting may make more new growth than the tree pruned in both years, although the former will have fallen behind the latter after a season's growth.

It appears that the first of these arguments is mainly concerned with the primary process of survival and the pruning treatment is advocated for the express purpose of aiding the tree to re-establish itself. In other words, under rigorous weather conditions a transplanted tree is less likely to die if pruned than if unpruned. The reason for this appears in the observed facts of date of leafing and of the quantity of leaves produced in the two cases. The transplanted tree must develop a new system of absorbing roots before it can meet the demands of the head for water, and it is therefore desirable to delay leaf development as long as possible in order to procure a large margin of safety between the demands of the new leaves and the capacity of the new absorbing system to supply water. Austin's work, to which reference has already been made (2), has given some quantitative data showing the smaller leaf area of pruned trees (not transplanted) early in the season.

It is not intended to convey the impression here that shoot-growth and root production are independent, but only that early in the season a considerable quantity of leaves may develop in the absence of corresponding new root-growth, a phenomenon which is familiar in hardwood cuttings and in trees suffering from root damage.

The recommendation not to prune transplanted trees until after a season's growth appears to be concerned with the amount of growth subsequently made rather than with the actual survival of the tree and, indeed, mortality among transplanted trees is uncommon enough to make subsequent growth a first consideration. If the tree is making good growth before lifting and if conditions are conducive to recovery (a question on which it is unfortunately difficult to express an opinion in advance) it is held that better results can be obtained by postponing pruning, since this will result, as the present experiments have shown, in the formation of a larger root system in the first season, with a corresponding effect upon subsequent growth.

In the case of older trees, such treatment may result in an excessive production of fruit buds during the first season which may result in over cropping in the second year and consequent lack of vigour. Even if removal of flowers is resorted to and actual fruiting thus reduced to a reasonable amount, the actual formation of bloom buds and the partial development of flowers naturally consumes some of the reserve products of the plant, though the loss may not be sufficiently large to have any considerable effect on growth.

A study of the influence of pruning immediately following transplanting was made by Chittenden (3), using several varieties of apple on " crab " and "paradise" stocks. His results showed that in the first season the pruned trees made considerably more growth (expressed as the length of new shoots developing) than the unpruned series, but in the following years the relationship was somewhat altered. The trees on " paradise ", pruned at planting, continued to make more growth than the unpruned trees but the difference was progressively less in successive years, the tendency being more marked in trees on "crab" stock. The fact that pruning, after the first year, was adapted somewhat to the needs of the trees may have contributed to this result. Chittenden's general conclusion is that in view of the importance of obtaining new shoot-growth for the formation of the general framework of the tree it is better to prune in the year of planting.

Whilst the trees in the present experiment were young and it is perhaps not permissible to compare them closely with older transplanted trees it is a fair assumption that in the case of the latter, pruning will reduce subsequent root-growth and the total weight of new growth, even though new extension growth is increased.

Pruning at transplanting certainly produces extension growth from which the framework of the tree can further be built up, but this occurs at the expense of root formation and of total growth, which may have a cumulative effect in later years. If the tree is left unpruned at planting a larger root system will result but extension growth will be sacrificed and it is doubtful whether its loss is rectified later on, even with the help of the larger root system.

General rules for practical guidance cannot be laid down until the investigation has proceeded much further. For example it is necessary to follow in detail, beyond the first year, the performance of trees pruned to different degrees in the year of transplanting.

SUMMARY.

Experiments have been carried out on an extended scale in order to amplify the results obtained in a previous investigation on the influence of winter stem pruning on subsequent stem and root development. Some five hundred trees were used, including various combinations of stock and scion and these were subjected to several different degrees of stem pruning.

Confirmation was obtained of the results previously recorded that the subsequent production of new roots is decreased and that of new shoots is increased by pruning, unless the treatment is very severe, in which case new shoot production may also be decreased. The age and condition of the buds which are induced to develop by pruning appear to influence the weight of new shoots produced.

In addition it was found that pruning decreases the thickening of old stems so much that any increase in new shoots is more than counterbalanced and thus total stem-growth shows a decrease.

Pruning diverts a proportion (depending on the severity of treatment) of the effort, normally directed to thickening of old stems, to the production of new shoots.

Although stem pruning has a considerable influence on the size of the root system, there is no evidence of a change of root character.

The ratio of new stem-growth to new root-growth remained remarkably uniform under all conditions of pruning irrespective of large changes in the actual amounts of both stem and root.

It is suggested that after transplanting although postponement of pruning until the second year will result in greater increment of both roots and total stem, if considerable extension growth is required, even at the expense of obtaining the maximum of new roots, immediate pruning is desirable.

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BOTRYTIS DISEASE OF LETTUCE

By M. M. ABDEL-SALAM, B.Sc., Ph.D.

A. INTRODUCTION.

THE writer's attention was first drawn to the subject of *Botrytis* disease of lettuce by the severe damage done by it to an early crop of this plant in the Hounslow district of Middlesex. This was in March 1930. Certain preliminary observations were made on this diseased material, but it was obvious that, as regards at least the attempted control, experiments should be begun in the Autumn. In order to prepare the ground and to familiarize himself with the nature of the disease the writer took in hand the study of its incidence throughout the season. Successive crops of lettuce were therefore grown under a variety of conditions throughout the Summer, and as will be shown later, preparations were made for attempting to control the disease in its serious Spring phase.

B. HISTORICAL REVIEW AND OCCURRENCE.

While one would rather suspect that *Botrytis* would occur on lettuce more or less wherever that plant is grown, it is curious that practically no mention of the disease is made in the current journals from European sources. The vast majority of references are from America and these will be dealt with first.

The first record of *Botrytis cinerea* on lettuce was in 1892 from Massachusetts when Humphrey (10) described a lettuce rot caused by *Botrytis* or *Polyactis*, which he concluded to be with little doubt the conidial stage of some fungus of the genus *Sclerotinia*. Jones (11) in the same year recorded a similar disease from Vermont, while Galloway (7, 1894) stated that the wet rot of lettuce due to *Botrytis* became serious with excessive humidity. Bailey (2) in his paper on fungi injuring lettuce published in 1895 attributed the lettuce rot to *Botrytis* which he regarded as a fungus living saprophytically on vegetable débris and becoming parasitic under favourable conditions. Selby (16, 1896) mentioned complaints by growers of the destructiveness of the lettuce rot or blight caused by *Botrytis vulgaris*. He recommended cultural sanitary treatment as a means of control because of the danger of using fungicides on edible leaves. Garman (8, 1899) referred to lettuce rot as being troublesome in Kentucky on lettuce growing under glass during the Winter and early Spring. He attributed it to several organisms including *Botrytis*. He believed that moisture on the leaves or in contact with them appeared to be a necessary condition for the development of the rot and suggested as a control measure that the exposed surface of the plants should be kept as dry as possible. Stone (18, 1900) published, after five years' observations, a paper on the "drop" of lettuce in which he described the following phases of the attack: 1, damping-off; 2, rotting of the plant soon after setting out; 3, dying of the lower leaves and "Black Roots"*; 4, rotting of the stems of mature plants; and 5, attack on the leaves of mature lettuce heads. He was of the opinion that the disease was carried out to the fields with transplanted seedlings in a more or less dormant condition and that it was because of the check due to transplanting that the fungus got the upper hand.

The drop and rot of lettuce was attributed since Humphrey's days to *Botrytis vulgaris* but in 1900 Smith (17) distinguished two types of drop in lettuce, namely a type due to *Botrytis cinerea* and another due to *Sclerotinia libertiana*. He was able to prove the pathogenicity of the two fungi by infection experiments and confirmed Kissling's observations (12) that conidia of *Botrytis* were unable to attack living tissue except after previous saprophytic nourishment. Newton (15, 1908) stated from preliminary investigations that the bacteria associated with the slime rot of lettuce may be secondary to injury caused by *Botrytis cinerea* and tipburn.

Beattie (4, 1924) described diseases of lettuce growing in greenhouses, including that caused by *Botrytis*. In the same year Heald and Dana (9) stated that *Botrytis cinerea* is one of the commonest species of the genus in the State of Washington, and listed a number of hosts including lettuce on which it caused "Black Root" and "Leaf Blight". Beach (3, 1926) showed that the alternation of great temperature extremes combined with rapid succulent growth was most important in predisposing lettuce to infection in cold frames. In the same year Melchers (14) found that the fungus attacking lettuce heads was easily transferable to cultivated geraniums in the greenhouse and conversely. Leach and Gilbert (13, 1926) stated that grey-mould due to *Botrytis* sp. is almost as destructive as the drop caused by *Sclerotinia* and under certain conditions may be more so. They added that it is more dependent on weather conditions than drop, and recommended strict sanitation and crop rotation as the only method of control known.

Weber (19, 1928) stated that grey-mould is the commonest of all diseases on lettuce and may cause considerable losses in transit.

As regards references to the control of the disease by use of fungicides there is little of importance to mention. Beach (l.c) found that in 1922 the application of formaldehyde to the soil caused considerable reduction of the disease but that there was no such favourable result when the experiment was repeated two years later. He was of the opinion that formaldehyde produced a stimulation of growth which rendered the lettuce more susceptible to frost and sun injury and hence to infection by *Botrytis*. He also showed that certain mercury compounds, in quantities not injurious to lettuce, gave no control of the *Botrytis* disease.

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* This is a misnomer as the root is not attacked. The disease is a typical collar-rot.

In the British Isles *Botrytis cinerea* has been reported as attacking a wide range of plants—many types of vegetables, ornamental shrubs and even trees—but as in the case of the Continent of Europe references to this fungus on lettuce, either in the current journals or in reports of experimental stations, are extremely scarce. On the other hand *Botrytis* disease of lettuce has been listed every year since 1918 in the Annual Reports of the Ministry of Agriculture (1). So far the only parts of England from which no records are forthcoming are the extreme South-West, East Anglia and the North-East. It is more than likely that the disease occurs there too, but has for some reason or another failed to attract attention. The amount of damage done was apparently very great in some cases, both under glass and in the open field.

As will be seen later, the most serious phase of the disease is where it attacks the stems of young seedlings, especially at the time when they are transplanted from the frames to the field in the Spring. The growers in the Thames Valley apply the name " Red Leg " to this phase of the disease. In spite of the fact that in some years great losses are incurred—up to 80 per cent. of the plants set out—the name " Red Leg " does not, to the writer's knowledge, occur in any English publication.

C. OBSERVATIONS ON SEASONAL OCCURRENCE.

The work to be described in this and the following sections was carried out chiefly at the Biological Field Station of the Imperial College of Science at Slough, Bucks. Some experiments were carried out from time to time in the laboratory at South Kensington, at the Chelsea Physic Garden and at a farm in Hounslow.

The present section deals with a series of successional plantings or sowings of lettuce throughout the year, beginning from the Spring of 1930. The amounts of natural infection, symptoms shown, etc., will be described for each case.

Experiment 1.—This was with a batch of seedlings (var. " Lobjoit's Cos ") obtained from a grower in Hounslow. These plants had been sown in frames in the usual way in the previous Autumn. The first planting-out at the beginning of March had largely failed on account of a serious attack of *Botrytis*. A sample of the seedlings still left in the frames was brought by the writer to Slough for experimental work. These were sorted into three classes according to the degree of attack as follows :

Class 1. Seedlings with stems devoid of lesions though *Botrytis* mycelium and conidia were frequently present on the dead and shrivelled lower leaves.

Class 2. Seedlings with reddish-brown shallow scars at the point of attachment of dead disintegrated lower leaves. These scars in some cases encircled the stem.

Class 3. Seedlings with large stem-lesions extending over a considerable area or running deeply into the stem tissue (Plate I, Fig. 1).

On leaving a few plants of the above classes under moist bell jars the dead areas were seen to become covered within twenty-four hours with *Botrytis* conidia, thus proving the presence of the fungus in the dead leaf bases and stem lesions. The rest of the seedlings were planted out in the field where they were kept under observation to the middle of May 1930. Data showing the degree of mortality due to *Botrytis* are given in Table I.

TABLE I.

Class.	No. of plants transplanted.	No. of plants killed by *Botrytis*.	% Mortality.
1	39	15	38
2	85	42	49
3	10	7	70
Total	134	64	48

The surviving plants showed totally healed lesions ranging from mere shallow dried-up cracks to deep pit-like depressions on the surface of the stem (Plate I, Fig. 2).

Though the number of plants in Class 3 was small, there was an indication that the degree of mortality ran parallel to the intensity of attack at the time of planting-out. Nevertheless the important point should be noted that heavy loss (38 per cent.) occurred in seedlings which to the practised eye would be judged free from disease at that time. It will be shown later that in such apparently sound plants the centre of infection is the dead leaf bases. The average mortality in this experiment (about 50 per cent.) was approximately that which the grower experienced—an illustration of the seriousness of this disease.

The progress of attack was as follows. The lesion originally present at the time of planting began to enlarge and new ones to appear at about ground level. The first obvious symptoms were the flagging of the outer leaves about a week after transplanting. At about the same time all the leaves lost their shiny appearance and became yellowish. Meanwhile the lesion continued to advance round and up the stem. Above the actual lesion the leaf bases showed a characteristic reddish-brown discoloration. By the time this stage had been reached the stem was disintegrated to such an extent that the slightest pull broke the aerial part from the root. In some cases rotting in the stem proceeded so rapidly in a horizontal direction that the whole plant fell over by its own weight before any drooping of the younger leaves had taken place.

Experiment 2.—Seedlings of " Lobjoit's Cos " and " Trocadero " growing in frames since early Winter were planted out in the field on April 24th, 1930.

Much less attack took place in this case as shown in Table II, which gives the condition of these plants when examined in June, at which time they were mature.

TABLE II.

Condition.	Cos.	Trocadero.
1. Healthy and free from lesions	20	8
2. Healthy but with healed lesions on leaf bases	9	23
3. Plants as a whole healthy but with active lesions on leaf bases	0	7
4. Plants healthy but with healed lesions on collar	5	0
5. Dead	5	0
Total	39	38

With both varieties the lesions referred to in groups 2 and 3 of the preceding table were confined to the lower leaves where they touched the ground. All the plants which were living at the end of the experiment were free from noticeable lesions on the head and were thus marketable.

Of the five Cos plants which died, two at least were attacked by insects so that it is not certain that *Botrytis* was the primary cause of death. One of the others is illustrated in Plate I, Fig. 3. This shows a very dark almost black lesion 2½ inches long encircling about half the stem. This is no doubt the phase known in America as " Black-root ". The tissue of the lesion was of a leathery nature due to plectenchyma formed in it. All round the edge as many as six reddish-brown sclerotia were found. These projected from the surface and characteristically pointed downwards. They were of different sizes and shapes varying from a slightly raised to a club-shaped body, about 6 mm. long, and appearing warty under a pocket lens. The surface of the lesion especially near the edge was sparsely covered with grey conidia.

The typical drooping of the foliage shown when a lettuce plant is in process of being killed by this disease is illustrated in Plate I, Fig. 4.

Experiment 3.—Seeds of " Lobjoit's Cos " and " Trocadero " were sown in the open on April 24th, and subsequently thinned so as to stand 1 foot apart. Some of the seedlings were also transplanted to fresh ground. No mortality took place in either of the two plots. The first indication of the presence of *Botrytis* was in the third week of June during a lengthy spell of hot weather following heavy rain-fall, when orange coloured lesions appeared on the centre of the midrib of the basal leaves of a few Cos plants. In all observed cases the lesion appeared on the surface facing the sun, suggesting that attack followed sun injury. The mesophyll of such leaves was water soaked to a distance of 1 cm. round this soft lesion. The tendency of these lesions was to expand more down than up the leaf, but by the third day they dried up. These plants matured by the middle of July. Their condition when then examined is shown in Table III.

TABLE III.

Condition.	Cos (316 plants).	Trocadero (115 plants).
Healthy and free from lesions	210 (67%)	0 (0%)
Healthy head but base of outer leaves slightly attacked	88 (28%)	98 (85%)
Lower leaves badly attacked and covered with conidia	4 (1·3%)	6 (5%)
Margin of wrapper leaves of head attacked but interior of head healthy	13 (4%)	8 (7%)
Head badly attacked and damaged	1 (0·3%)	3 (2·5%)
Dead	0 (0%)	0 (0%)
Total plants attacked	33·5%	100%

The results shown in Table III are similar to those of Table II up to a point. Thus there was a higher percentage of perfectly healthy plants in the Cos than in the Trocadero variety, due no doubt to the spreading habit of the latter (Cabbage) type. The great majority of the latter plants showed lesions on the lower leaves only as was also the case in Table II. In this experiment there were no dead plants at all, but a new phase of the disease appeared, viz. attack on the upper leaves of the head. In this respect the Trocadero variety was sowewhat more affected than the Cos. The attack started at the margin of the wrapper leaves, which became water soaked and light brown in colour. In extreme cases this soft rot extended from the outer leaves inwards till the whole head was one-sided. The rotted parts showed abundant development of *Botrytis*, either as conidia or as sclerotia according to the strain of the fungus.

Experiment 4.—Seeds of the varieties used in the previous experiments were sown on May 29th, 1930, and similarly thinned out, some of the seedlings being transplanted to fresh ground. No collar-rot appeared on any of the seedlings. The plants were examined when matured in the middle of August. Their condition is given in Table IV.

TABLE IV.

Condition.	Cos.	Trocadero.
Healthy and free from lesions	127 (69%)	0 (0·0%)
Base of lower leaves slightly attacked but head free from *Botrytis*	46 (25%)	266 (97·8%)
Shallow healed lesions at foot of stem but head healthy	6 (3·3%)	0 (0·0%)
Wrapper leaves attacked but head healthy	5 (2·7%)	0 (0·0%)
Head badly attacked	0 (0·0%)	6 (2·2%)
Total	184	272

These results are similar to those in Table III. It should be added that the heads were only attacked near the time of maturity.

Though the great majority of heads were free from *Botrytis* more than half of them showed a bad attack of the trouble called "Greasiness" by the growers in the Thames Valley. This is a serious disease causing a considerable loss of plants just at the time when they are about ready for market. It would appear to be similar to, if not identical with, the condition known as "Tip-burn" in American literature. Although the disease appeared rather later in 1930, viz. in August, it was already pronounced in June of the following year, being shown in not less than 80 per cent. of mature Trocadero lettuce heads in experimental plots at Slough. It was also reported by growers in the same district as causing serious damage to mature lettuce. Since *Botrytis cinerea* may occur on greasy heads as a secondary parasite it is important to distinguish between the two diseases.

The features of "Greasiness" are as follows:

1. It invariably begins at the leaf margin, from which it proceeds inwards to the veins. At the earliest stage the finer veins of the leaf margin turn reddish-brown, assuming a network appearance. Later the mesophyll cells nearest the discoloured veins become water soaked, soft and dark brown in colour, in contrast to the reddish-brown caused by *Botrytis*. This condition, as the disease advances, involves the whole mesophyll between the discoloured veins (Plate I, Figs. 5 and 6). Meanwhile the discoloration works its way down the stouter veins with subsequent degeneration of the mesophyll between them. The final stage is attained when the whole blade disintegrates into brownish slime which drips down inside of the head. The attack is not restricted to the wrapper leaves but is deeply seated in the head as well. Thus when the disintegrated wrapper leaves are removed, earlier stages may be seen as the more deeply seated leaves are exposed.

The disease may be checked when weather conditions unfavourable to its spread set in. Thus at the earliest stage the disintegrated margins of two overlapping leaves may dry up and stick together, appearing like light brown semi-transparent parchment. At a later stage the more or less disintegrated leaves may dry up, forming a shrivelled blackish net round the head.

2. *Botrytis* spores and conidiophores are absent, at least in the earlier stage. Numerous bacteria are present.

3. The cause is believed to be physiological, the predisposing factor being the occurrence of a hot dry spell after a period of wet weather. Certain workers on the other hand have claimed a primary role for bacteria (*B. aptatum*) in this connection.

Experiment 5.—Seeds of seven varieties of lettuce were sown on August 5th, 1930, in boxes kept outdoors. On September 8th these seedlings were examined with the results shown in Table V.

TABLE V.

Variety.	Total No. of seedlings.	No. showing Collar-rot.	% Attack.
Cos.			
Bath's Black-Seeded ..	252	0	0
Hicks' Hardy White ..	203	5	2·5
London Superb White ..	264	30	11·4
CABBAGE.			
Continuity	119	7	5·9
Lees Immense	176	9	5·1
Trocadero	220	23	10·4
All the Year Round ..	203	33	16·2

Taking the Cos and the Cabbage varieties as a whole, there was more attack in the latter than in the former. With both types there appeared to be some varieties which were more affected than others.

The seedlings with no obvious lesions were planted out on September 9th, some in the field, some in boxes outdoors and some in cold frames. A few of these plants succumbed but the blanks were filled up with healthy plants and observations continued throughout the Winter. Table VI gives a record of the numbers of the plants which died month by month.

TABLE VI.

Number of Plants Killed.

Month.	Cos varieties (90 plants).	Cabbage varieties (186 plants).
October	0	0
November	3	3
December	7	13
January	8	26
February	22	40
March	3	12
April	4	9
Total killed	47	103

The salient feature brought out by Table VI is the high mortality which occurred in February. This being the coldest month of the year, one was lead to associate the increased incidence of *Botrytis* disease with the low temperatures prevailing. One saw in fact that severe attacks of *Botrytis* always followed frost periods, so that it was legitimate to assume that under those conditions the fungal disease was secondary to frost injury.

One may summarize the course of events described in this section as follows :

1. The so-called " Red-leg " is the most serious phase of *Botrytis* disease in lettuce, often causing high mortality when the plants which have been overwintered in frames are planted out in the open ground in the early Spring.

There is also some mortality in autumn sowings in the open, but none on sowings made in the open during late Spring and Summer.

2. The disease in the form of lesions on stems and main veins of the lower leaves is common throughout the season, but during the Summer the damage is unimportant and affected plants are quite saleable. Cabbage varieties, probably because of their habit of growth, are more liable to such injuries than Cos varieties.

3. Attack of upper leaves is confined to the summer months and appears to be related to sun-injury. This may take the form of isolated spottings or the whole leaf may be involved.

4. Damage in the form of stem-rot is frequent on lettuce growing in the open over Winter and is, to some extent at least, secondary to frost injury.

D. EFFECT OF CULTURAL CONDITIONS ON AMOUNT OF DISEASE.

As was pointed out in the preceding section, the most serious phase of *Botrytis* disease is shown when lettuce which has overwintered in frames is planted out in early Spring in the open. Observations at Hounslow and Slough and the statements of growers indicate that the treatment of the plant in the frame, the conditions prevailing in the open at the time of planting, and the precise method of planting have some influence on the amount of disease which develops. Factors which arise in this connection are as follows:

1. Date of sowing in the Autumn.
2. Date of planting out in the Spring.
3. Method of planting, whether deep or shallow.

These will be dealt with in order.

1. DATE OF SOWING IN AUTUMN.

An illustration of the effect of early sowing was seen in a series of outdoor frames of the usual kind at a farm in Hounslow. Three of these had been sown on October 8th, 1930, but the great majority on October 18th and 25th. The writer examined these frames on March 6th, 1931. The earliest sown frames showed a remarkable development of *Botrytis*, about 75 per cent. of the plants being dead and the whole frames being rampant with conidiophores of the fungus. The surviving plants showed varying degrees of collar-rot. In the main sowing on the other hand it was only on close observation that small localized patches of plants killed by *Botrytis* could be found. The vast majority of the seedlings when looked at from above appeared to be perfectly healthy. The appearance of the earliest sown plants was so bad that the grower was on the point of scrapping them. A batch of these and of the seedlings sown on October 18th were brought to Slough for further test. It was intended to plant these out in the open in the usual way but on account of frosty weather, they were all heeled-in in a greenhouse for one week. At the end of this time examination showed the condition given in Table VII.

TABLE VII.

Date of Sowing.	Total No. of seedlings.	No. of seedlings killed by *Botrytis*.	% Mortality.
October 8th	240	145	60·4%
October 18th	220	95	43·2%

There was thus a heavy mortality, greater in seedlings of the earlier sowing but not remarkably so. This was borne out in a further test in which a fresh lot of seedlings of October 8th, 18th and 25th, 1930, were brought from the same frames of the Hounslow farm and planted out at Slough on the same day. The amount of mortality over a period of thirty days is shown in Table VIII.

TABLE VIII.

Date of Sowing.	Total No. of seedlings.	No. of seedlings killed by *Botrytis*.	% Mortality.
October 8th, 1930 ..	105	74	70·5
October 18th, 1930 ..	180	108	60
October 25th, 1930 ..	80	39	49

It appears then that abnormally early sowing increases the incidence of *Botrytis*, but it is also clear that sowings at the normal time give a deceptive appearance of freedom from the fungus inasmuch as a high percentage of seedlings succumb when planted out in the field. A similar result has already been mentioned in Experiment 1 of the preceding section where seedlings with stems devoid of lesions gave 38 per cent. of mortality when planted out in the field.

2. DATE OF PLANTING OUT.

It is a general experience of the growers that mortality due to *Botrytis* is higher in seedlings planted out in the field early in the Spring than in those later. The following experiment shows this to be the case. Two batches of Lobjoit's Cos seedlings both sown on October 18th, 1930, and grown at the same farm in Hounslow were planted out at Slough on March 13th and April 22nd, 1931, respectively. The result of the experiment is given in Table IX.

TABLE IX.

Date of Planting.	Total No. of plants.	Total No. of seedlings killed.	% Mortality.
March 13th, 1931 ..	160	64	40
April 22nd, 1931 ..	197	47	24

3. METHOD OF PLANTING, WHETHER DEEP OR SHALLOW.

To find out whether deep or shallow planting affected in any way the development of *Botrytis* disease, seedlings of Lobjoit's Cos from the same farm at Hounslow were planted out at Slough, some with their stems so deeply sunk in soil that the bases of the lower leaves were covered, the rest with their roots only covered. The stems of the latter lay flat on the ground because of the weight of the leaves. The results of the two experiments in this connection are shown in Table X. In each experiment forty plants were used for both the deep and shallow plantings.

TABLE X.

Expt.	Number killed.	
	Deep planting.	Shallow planting.
1	20	27
2	15	24

It does not appear from the results shown in the above Table that deep planting aggravates the disease, as was the opinion of the grower from whom the plants were obtained. On the contrary, in both experiments there was a somewhat lower mortality in the case of the deeply planted plants. The idea of the grower in question was that, with deep planting, the collar of the plant is kept moist and is therefore rendered more liable to attack. This argument seems sound, but is not borne out by the facts in the present case. It is possible that in a Spring with dry overhead conditions the results might be different.

E. ISOLATION OF THE FUNGUS.

1. METHOD OF ISOLATION.

This depended on the nature of the material. When conidia were present in abundance on the host surface these were generally dusted on to nutrient agar. When conidia were absent, a piece of the diseased tissue was cut after sterilization of the surface and transferred to nutrient agar. Subcultures were made on plain agar in order to eliminate bacterial contamination, Brown's method (6) being used. Altogether eighty-six isolations were made throughout the year from which thirteen strains of *Botrytis* were sorted out on the basis of differences in macroscopic character when grown on Czapek's agar in Petri dishes. Brown's hyphal tip method (6) was used for their purification.

2. STRAINS.

These strains fall into two main groups, one of which is characterized by the profuse development of sclerotia and comparative rarity of conidia, the other which shows abundant development of conidia with few or numerous sclerotia. As only two strains, A and B, have been used in this work for purposes of inoculation, the detailed mycological features of the strains as a whole will not be described in this paper. Strains A and B are both of conidial type, differing from each other chiefly in the shape of the spore, as shown in Table XI.

TABLE XI.

	Strain A.	Strain B.
Shape.	Ovate oblong.	More round than oblong.
Range of dimensions ..	6·0—15·0×5·0—9·2μ	5·0—13·2×5·0—10·0μ
Mean of 100 measurements ..	11·1×7·25μ	9·7×8·0μ

F. EFFECT OF ENVIRONMENT ON ATTACK.

It has been shown in sections C and D that the severest losses due to *Botrytis* occurred during the Winter and early Spring. Since low temperature, high humidity and poor light are factors prevailing at these times of the year, one would expect these to favour the disease. No exact data bearing on these relationships have been obtained in this work as the necessary elaborate apparatus has not been available.

The results obtained in an experiment carried out at the Chelsea Physic Garden appear to indicate that low temperature favours the attack of lettuce by *Botrytis*. Seedlings of Lobjoit's Cos and Trocadero lettuce of autumn sowing were transplanted into boxes of soil on March 2nd, 1931, and inoculated on the same day with strain B of the fungus, using two methods :

1. spraying seedlings with a dense suspension of conidia.
2. putting a fairly heavy inoculum of mycelium and conidia into a wound at the base of the stem.

Each of these had a control similarly treated except for the omission of the fungus. These boxes were then divided between an outside frame (temperature ranging from 1° to 15° C.) and a greenhouse heated to 18° C. All the boxes were plentifully watered throughout the course of the experiment, so that one may provisionally consider the differences in results as being effects of the temperature. The data obtained are shown in Table XII.

It will be noticed from the Table that there was a considerable amount of natural infection in the control (and therefore presumably also in the inoculated) plants. In fact there was less infection in the spray-inoculated Cabbage Trocadero seedlings in the cold frame than in the control. The efficiency of inoculation, especially by spraying with spores, is therefore open to doubt, but the experiment clearly indicates that infection, whether natural or artificial or a combination of both, is much less in the warm greenhouse than in the cold frame.

TABLE XII.

Condition of Experiment.		Plants infected (%).	
		Lobjoit's Cos.	Trocadero.
A. Wound Inoculation.			
Outdoor frame	Inoc. ..	40	100
	Control ..	27	52
Warm Greenhouse	Inoc. ..	0	48
	Control ..	0	0
B. Spray Inoculation.			
Outdoor frame	Inoc. ..	47	52
	Control ..	33	68
Warm Greenhouse	Inoc. ..	0	8
	Control ..	7	0

As regards the effect of humidity, the writer's observations are in a general way in agreement with the view that this factor favours attack. Thus the fact that the cabbage type is more susceptible to attack of the basal leaves than is the Cos type may be correlated with the spreading habit of the former which tends to preserve a humid atmosphere round the leaf bases. Observations in the field showed that in a spell of wet weather attack was liable to occur on all parts of the plant. Immediately dry weather came on, lesions on exposed parts of the leaves quickly dried up and the attack was arrested. On the other hand the disease progressed for a long time on less exposed parts, e.g. on leaf bases of the Cabbage type of lettuce, by which time the soil might be relatively dry. This suggests therefore that atmospheric humidity is probably more important to the progress of attack than is the percentage of water in the soil.

This view receives further support from the observations cited on p. 23 where it was stated that 75 per cent. of the plants in a frame had succumbed to *Botrytis* attack. This frame had received no overhead watering during the whole Winter. The surface layer of soil was in consequence quite dry and powdery. Nevertheless there was very severe attack.

G. VARIETAL RESISTANCE AS SEEN FROM NATURAL INFECTION.

It has already been seen, e.g. in Table V, that some varieties both of Cos and Cabbage types appear to be more susceptible to *Botrytis* attack than others. A more extended test on a number of varieties with reference to this point is contained in the present section. The following ten varieties were used :

Cos type : Hicks' Hardy White, Bath's Black-Seeded, London Superb White, Lobjoit's Cos.

Cabbage type : Trocadero, Lee's Immense, All The Year Round, Continuity, Feltham King, Glory of Nantes.

Experiment 1.—Seeds of these were sown on September 25th, 1930, on formaline-sterilized potting soil in boxes in a cool greenhouse which contained Geranium cuttings heavily infected with *Botrytis*. The seedlings were left crowded in the boxes all through the experiment in order to ensure optimal conditions for infection. By the end of November all but the following varieties were entirely destroyed by *Botrytis*: Lee's Immense, Bath's Black-Seeded, Hicks' Hardy White, Feltham King and Glory of Nantes. The first-named variety showed no attack at all; in the remainder the degree of attack varied from 5 to 10 per cent. By the end of December the last two varieties also succumbed. The remaining three when examined on February 16th, 1931, gave the results shown in Table XIII.

TABLE XIII.

Variety.	Total No. of plants.	No. of plants killed by *Botrytis*.	% Attack.
Lee's Immense	360	100	28·6
Bath's Black-Seeded ..	297	171	57·5
Hicks' Hardy White ..	258	105	40·6

Experiment 2.—Seedlings of five varieties were planted out on September 21st, 1930, in an outdoor frame and were treated in the usual way. The amount of attack by April 28th, 1931, is given in Table XIV.

TABLE XIV.

Variety.	Total No. of plants.	Plants killed by *Botrytis*.
Lee's Immense	24	2
Hicks' Hardy White ..	24	6
Bath's Black-Seeded ..	24	7
Continuity	24	10
Trocadero	24	11

As in Experiment 1, the least susceptible to attack was Lee's Immense. Hicks' Hardy White and Bath's Black-Seeded came next and showed much the same degree of susceptibility. Continuity and Trocadero were the most susceptible.

Experiment 3.—In an experiment on frost resistance certain frost-hardy varieties were found to be the least attacked by *Botrytis* as shown in Table XV. The seedlings in this experiment were planted out on September 9th, 1930, and the final examination was on April 28th, 1931.

Thus under the different conditions of these experiments there was general agreement that among the varieties tested the least susceptible to *Botrytis* disease was Lee's Immense followed by Bath's Black-Seeded and Hicks' Hardy White.

Table XV.

Variety.	Total No. of plants.	No. of plants killed by *Botrytis* following frost.	% Attacked.
Lee's Immense	63	16	25·4
Bath's Black-Seeded ..	30	9	30·0
Hicks' Hardy White ..	30	13	43·3
All The Year Round ..	63	46	53·0
Trocadero	30	28	93·3
London Superb White ..	30	30	100·0

H. MECHANISM OF RESISTANCE.

It has already been stated in Section C that in some cases lettuce plants will come to maturity in spite of attack by *Botrytis* at an earlier phase of their growth. A striking example of this behaviour has been shown in Plate I, Fig. 2, illustrating a plant which was badly attacked at the time of transplanting but which nevertheless recovered and grew to maturity. The active lesion had definitely healed up. Similar cases have been observed throughout the season, more especially during the Summer months. The lesions on such recovered plants vary from shallow scars to deep pit-like depressions.

An actively advancing lesion has a shiny ochraceous appearance and the affected tissue is soft and discoloured, even beyond the region invaded by the fungus. This discoloration is specially marked in the walls of the vascular strands. Occasionally latex may be seen oozing from the surface of such lesions. In cases where the advancing lesion has gone so far as to kill the plant, the invaded tissue appears as a thin-walled somewhat crumbly or sponge-like mass containing abundant hyphae and incipient sclerotia. When from some reason the advance of the fungus is checked, the lesion takes on a darker colour—reddish-brown or in some cases almost black. The invaded tissue may remain as a hard collar round the stems, or may crack longitudinally owing to the growth of the tissue underneath and finally appear as sheets of hard brown tissue more or less embedded in green cortical tissue (Plate I, Fig. 7).

The relationship of the fungus to the host can be easily observed in unstained hand sections. The killed area consists of hard disintegrated tissue with abundant fungal hyphae and the numerous flake-like hard sclerotial masses. Separating this from the healthy living tissue is a wide band of cells which are free from fungal elements and which are discoloured brown and have thick walls. The appearance suggests that there is a deposition of gum-like substance on the cell walls. There is no evidence of cork formation. One may suggest, therefore, that resistance of the host tissue is correlated with the development of this infiltrated layer and that the problem of resistance to *Botrytis* in lettuce has similarities to that of certain varieties of plums to *Stereum purpureum* as outlined by Brooks (5).

I. CONTROL.

It has been shown in previous sections that " Red-leg " or Collar-rot is the most dangerous of all phases of *Botrytis* attack on lettuce. So serious are the losses experienced by the growers from the disease that effective measures should be directed to its control. As the greatest damage occurs when overwintered seedlings are transplanted out in the Spring such measures would naturally be on the following lines :

(a) Treatment of overwintered seedlings when pulled out at the time of transplanting.

(b) Treatment in the frames for possible improvement of frame culture with a view to the production of seedlings free from fungus.

(a) TREATMENT OF SEEDLINGS AT THE TIME OF TRANSPLANTING.

As it has been shown that overwintered seedlings carry *Botrytis* in the bases of their dead leaves and on lesions on their stems, the possibility was explored of eliminating some of this infection by a process of surface sterilization without causing injury to the seedlings. Two experiments were carried out to this effect.

Experiment 1.—Three batches of Lobjoit's Cos lettuce, sown respectively on October 8th, 18th and 25th, 1930 (and referred to as Class I, II and III respectively) were each divided into four lots and treated as follows :

1. Control.
2. Steeped for half an hour in 0·5 per cent. Uspulun solution, washed with water and planted out.
3. Dusted with Ceresan before planting.
4. Steeped for one minute in Bordeaux mixture and allowed to dry overnight before planting out.

Transplanting was on March 13th, 1931. The final result after thirty-eight days is shown in Table XVI.

The effect of the Uspulun treatment was to reduce the wastage of plants by approximately one-half. On the other hand the treatments with the other two fungicides proved to be definitely harmful. In the case of Ceresan the seedlings appeared healthy for some days but rapidly succumbed when the foliage was wetted by rain, presumably as the result of a burning action of the fungicide. Dipping of the plants in Bordeaux mixture with subsequent drying of the roots overnight was definitely harmful as the plants never recovered after transplanting.

TABLE XVI.

Treatment.	Class I.			Class II.			Class III.		
	Total plants.	Killed by *Botrytis*.	% Killed.	Total plants.	Killed by *Botrytis*.	% Killed.	Total plants.	Killed by *Botrytis*.	% Killed.
Control ..	51	44	86	80	43	54	40	20	50
Uspulun ..	50	26	52	80	21	26	40	10	25
Ceresan ..	51	51	100	80	80	100	40	40	100
Bordeaux Mixture ..	51	51	100	80	80	100	40	40	100

Experiment 2.—Seedlings of varieties Lobjoit's Cos and Trocadero sown on October 18th, 1930, were sorted into three classes:

Class I, plants apparently healthy.

Class II, plants with very shallow lesions at base of leaves.

Class III, plants with deeper lesions.

These were treated as follows:

1. Seedlings of Lobjoit's Cos were steeped in 0·5 per cent. Nu-green solution for one hour and planted out unwashed.
2. Seedlings of Trocadero were steeped in 0·5 per cent. Uspulun for one hour and similarly planted unwashed.

Each treatment had a control.

Planting out was on April 22nd, 1931, and after twenty-seven days the results were as given in Table XVII.

TABLE XVII.

Variety and treatment.		Class I.		Class II.		Class III.	
		Total plants.	% killed.	Total plants.	% killed.	Total plants.	% killed.
Cos	Control	116	17·2	52	25	29	45
	Nu-green	226	5·7	102	1·9	27	3·7
Trocadero	Control	30	3·3	108	7·4	52	21·1
	Uspulun	53	3·8	204	0·5	108	3·8

The effect of the Nu-green treatment, over all the three classes of plants, was to reduce mortality from 24 per cent. to less than 5 per cent. The corresponding figures for the Uspulun treatment were 10·5 per cent. and 2 per cent.

The much lower incidence of disease in this experiment is probably mainly due to the later date of transplanting.

(b) TREATMENT IN THE FRAMES.

The growers' practice for lettuce seedlings overwintered in frames is to allow of no overhead watering. The frames are banked up in the Autumn so as to face the South, and after the seedlings appear above ground, the lights are taken off on dry days but always kept on at night and in wet weather. As has been shown, however, these relatively dry conditions do not suffice to eliminate *Botrytis* invasion, even if the latter is mostly in a dormant form.

A possible line of control is the watering of the frames from time to time with a fungicidal solution. An experiment of a somewhat wider scope was carried out during the Winter of 1930-1, and although certain complications arose the results are of sufficient interest to be included here.

Frames of the usual kind were sown with Trocadero lettuce on November 15th, 1930, and given the usual commercial treatment. Unfortunately severe damping-off due to *Rhizoctonia Solani* set in before the end of the year. The surviving plants were pulled up in the middle of February and replanted (260 plants per frame) after the soil had been treated as follows :

Frame 1. One watering with 14 litres of 0·5 per cent. Cheshunt Compound immediately before replanting.

Frame 2. Three waterings with 14 litres of 0·5 per cent. Cheshunt Compound at ten-day intervals.

Frame 3. Two applications of 14 litres of 0·25 per cent. Uspulun solution at ten-day intervals.

Frame 4. One application of 14 litres of water as a control.

Though damping-off was not entirely suppressed in any of the frames a good crop of seedlings was carried through till the Spring. Unfortunately from the point of view of the particular experiment in hand, the plants in all the frames remained free from all visible signs of *Botrytis* disease.

A batch of plants from each frame was transplanted into the open ground on April 22nd, 1931. None of these plants succumbed to *Botrytis* disease in the sudden manner characteristic of the seedlings obtained from the Hounslow farm. A certain number began to die off later, presumably as a result of infection in the field. The plants treated with Cheshunt Compound and Uspulun actually showed more attack than the controls. Treatment with the former fungicide gave a very luxuriant type of seedling, no doubt as a result of its value as a fertilizer. On the other hand the soil treatment with Uspulun was harmful to the plants as they remained dwarfed to the end of the experiment. Thus while a short immersion of the seedlings in certain fungicides at the time of transplanting offers promise of success in the control of this disease, watering of the soil of the frames with fungicidal solutions during the growth of the plants appears to be definitely dangerous.

J. SUMMARY.

1. *Botrytis* disease of lettuce is widely distributed in England where it has been reported to cause serious losses.

2. Observations on the seasonal occurrence of the disease showed that the most serious phase of attack is the collar-rot, known locally to growers in the Thames Valley as " Red-leg ". This may cause a loss of as much as 70 per cent. of overwintered seedlings when planted out in the Spring. It also causes some loss in autumn sowings but none in sowings made in the open in late Spring or in Summer.

Botrytis disease in the form of lesions on the stem and on the veins and blades of the lower leaves is of frequent occurrence all through the Summer. Such injuries do not as a rule lower the marketable value of the plant. This type of injury is commoner on Cabbage than on Cos types of lettuce, probably on account of the spreading habit of growth of the former.

Attack of the upper leaves leading sometimes to " head-rot " occasionally occurs during the Summer. There is evidence that this type of attack is associated in some cases with sun-injury.

Lettuce overwintering in the open ground is frequently killed by *Botrytis* in the form of a stem-rot. Such attack is largely correlated with frost injury.

3. *Botrytis* occurs as secondary to what is known in the Thames Valley as " Greasiness ", a disease similar to that described as " Tip-burn " in America.

4. As regards the effect of cultural conditions on incidence of the disease, it appears that the latter is increased by abnormally early sowing in the Autumn. Seedlings which are sown at the normal time may give a deceptive appearance of healthiness, inasmuch as a high percentage of apparently sound plants may succumb when planted out in the Spring.

Overwintered seedlings when planted out early in the Spring show a higher incidence of disease than those planted out later.

5. Thirteen strains of *Botrytis cinerea* were sorted out from isolations made throughout the year. These fall into two distinct groups, one characterized by the profuse development of sclerotia with few conidia, the other with abundant conidia and few or numerous sclerotia.

6. Infection, whether natural or artificial, is much less in the warm greenhouse than in cold frames.

Observations showed that high humidity favours attack. It is suggested that atmospheric humidity is probably more important to the progress of attack than the degree of humidity in the soil, judging from the severe attack which may occur in frames with dry soil.

7. Of ten varieties of lettuce tested with regard to differential susceptibility, the Cabbage type Lee's Immense proved to be the most resistant, followed by the Cos types Bath's Black-Seeded and Hicks' Hardy White.

8. Active lesions of various depths may definitely heal up, the plant coming to maturity. It is suggested that resistance of the host tissue is correlated with the development of an infiltrated layer of a gum-like substance in the healthy tissue bordering the lesion.

9. Whereas steeping the overwintered seedlings at the time of transplanting in 0·5 per cent. Uspulun or Nu-green solutions for half to one hour gave promise of value in the control of the "Red-leg" phase of the disease, watering the soil of the frames with Uspulun during the growth of the seedlings was found to cause considerable permanent stunting of the plants.

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PLATE I.

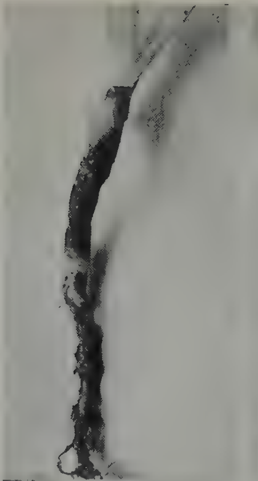
FIG. 1

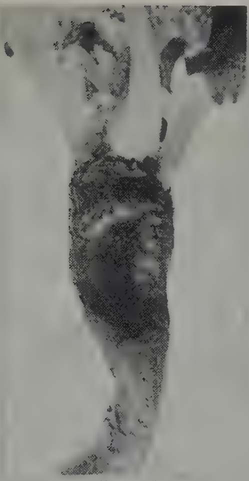
FIG. 2

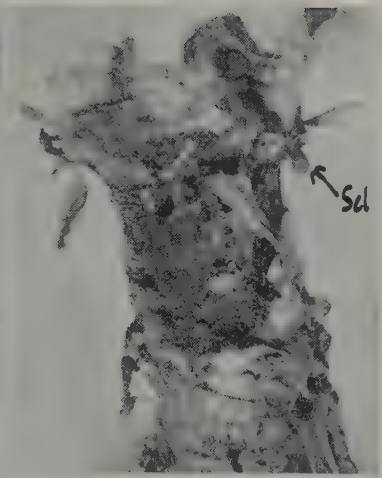

FIG. 3

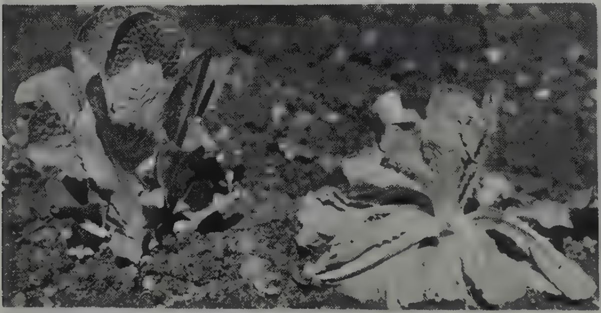
FIG. 4

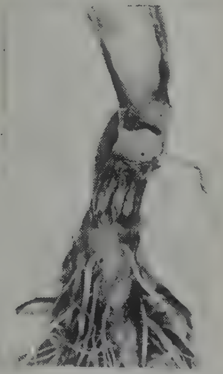
FIG. 7

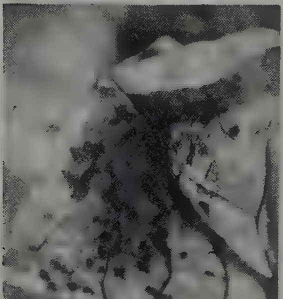
FIG. 5

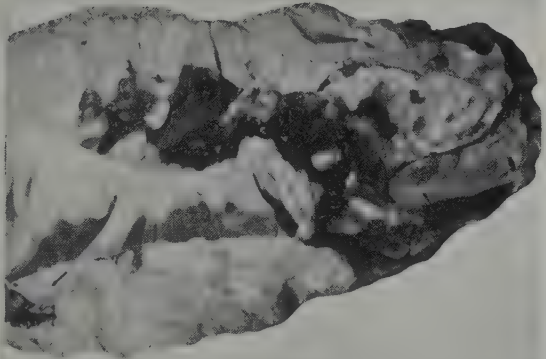
FIG. 6

FIG. 1. Overwintered seedling of Lobjoit's Cos lettuce showing large lesion of *Botrytis cinerea* on stem. (Slightly enlarged.) FIG. 2. Plant of Lobjoit's Cos with totally healed lesion. (Slightly enlarged.) FIG. 3. Mature Cos plant with large lesion containing Sclerotia (Scl.) of *Botrytis cinerea*. (Slightly reduced.) FIG. 4. Lobjoit's Cos plants: left, healthy; right, attacked by *Botrytis cinerea*. (Reduced.) FIG. 5. Part of leaf of Trocadero plant showing early symptoms of "Greasiness". (Natural size.) FIG. 6. More advanced stage of preceding. (Reduced.) FIG. 7. Young plant of Lobjoit's Cos with healed superficial lesion. (Natural size.)

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A COMPLEX EXPERIMENT IN THE PROPAGATION OF PLUM ROOTSTOCKS FROM ROOT CUTTINGS

SEASON 1931-1932

By T. N. HOBLYN
East Malling Research Station, Kent, England
AND
R. C. PALMER
Experimental Station, Summerland, B.C. Canada

I. INTRODUCTION.

THE desirability of propagating fruit tree rootstocks vegetatively is now becoming generally recognized. Unfortunately, some of those stocks which have been shown to exert the most beneficial influence upon scion varieties have themselves proved comparatively difficult to propagate. Accordingly, it is most important that a thorough investigation be made into every method of facilitating the vegetative propagation of fruit tree stocks.

Rootstocks have long been propagated from root cuttings by European nurserymen. In fact, very large quantities of the Common Mussel plum are propagated by this method in England. Recent investigations conducted by Upshall (6) and Yerkes (8) indicate that the root cutting method of propagation has commercial possibilities under American conditions. Nevertheless, a search of the literature reveals a scarcity of accurate scientific data regarding the influence of such factors as time of making, time of planting and size of root on the viability and subsequent growth of root cuttings. The responses of various kinds of rootstock to the root cutting method of propagation also merit investigation. Again, evidence is urgently needed regarding the comparative ease and economy of propagation by root cuttings as contrasted with propagation by other means such as stem cuttings and layerage.

Information regarding propagation by stem cuttings and layerage having already been rendered available by Knight and his associates (4, 5), the time seemed opportune for a comprehensive investigation of the possibilities of propagation by root cuttings.

The type of investigation undertaken also provided the means of testing the adaptability of certain methods of plot layout to the study of a complex horticultural problem.

II. THE PROJECT.

The main object of this experiment, which was done at East Malling during the season 1931-32, was to test the possibilities of raising rootstocks for plums from cuttings taken from the roots of older trees. There were, however, a number of problems connected closely with this line of research, and in this experiment an attempt was made to investigate as many aspects of the main problem as possible at the same time.

Thus, instead of eliminating all causes of variation, such as variety, time of planting and size of cutting, and trying to obtain a straight answer to a simple question with standard material, as has been rather the principle in older experiments of this nature, the trial was planned in such a way that a large number of questions, all of which were really a part of the main problem, could be answered by a single experiment.

The results of the experiment amply justify this method of approach, since it will be shown that success in this method of propagation was only possible by taking the best sized cuttings and planting them at the right time ; and that whereas success was possible with one variety, with another the method was a complete failure.

The following issues seemed of importance and were therefore included in the experiment.

(a) *Variety :* Two representative varieties of plum commonly used as rootstocks were chosen, the first Common Mussel, a variety which roots from cuttings, layers, etc., with comparative ease ; and the second, Pershore Egg, which is usually propagated with difficulty.

(b) *Time at which the cuttings were made :* In the original project the cuttings were to be made at monthly intervals from October 1931, to March 1932. Weather conditions, however, interfered in November so that this month had to be omitted and the series extended till April. There were thus six times of making : October 29th, December 13th, January 25th, February 27th, March 22nd, and April 15th.

(c) *Time of Planting :* Half of the cuttings were planted as soon as possible after they were made ; the other half were bedded in sand under cover until the Spring, when they were all planted out together ; the October and December batches being planted on April 9th ; the January, February and March batches on April 13th ; and both the April lots on the day they were made—April 15th.

(d) *Size of Cutting :* This problem has two aspects, (1) length, and (2) diameter. Two lengths of cutting were chosen, i.e. long cuttings, 12 cms. in length, and short cuttings, 6 cms. long. The cuttings were further divided into three grades for diameter, viz. 3-6 mms., 6-9 mms. and 9-12 mms. There were thus six different sizes ranging from a short thin cutting to a long stout cutting.

PLANNING THE EXPERIMENT.

In an experiment such as this, it is necessary to include all possible combinations of all the issues in the trial, since if one were to be omitted, that one might be the very combination which is being sought. Thus, short stout cuttings might stand the Winter better than long thin ones, so that if planted in October, the former might have given the better results, but if bedded in till Spring the reverse might have been the case ; and this might be true for one variety, but not for another.

Further, from the point of view of replication of like plots, if a number of unrelated treatments is selected, a comparatively large number of repetitions is required. If, however, all possible combinations are included, the number of replications can be greatly reduced. For example, in the present instance, if the two varieties are compared, there are seventy-two different treatments of each variety, if all combinations are included. If each of these treatments is only duplicated, the variation between each of these seventy-two duplications can be used for the estimate of error, when the varieties are compared.

In this experiment, therefore, there were originally 144 combinations of variety, time of planting, and size. These were made up of two varieties, six times of making, two times of planting (i.e. at once and in Spring), two lengths of cutting, and three grades for diameter.

SIZE OF PLOT AND NUMBER OF REPLICATIONS.

Very little information existed with regard to the size of plot and number of repetitions necessary in this kind of experiment, and the eventual size of plot was determined rather from convenience and available material than for any other reason. At the same time, since the most important result of this experiment would be the actual proportion of the cuttings to take root and grow, it was necessary to have sufficient cuttings in each plot to estimate this factor. Finally, it was decided to have ten cuttings in each plot and to duplicate the plots. Thus there being 144 treatments, there were 288 plots each containing ten cuttings. These were planted in a special cutting bed surrounded by boards.

The cuttings were planted 2½ inches apart in the row, the rows being 8 inches apart. There being two plots to a row, eleven cuttings were planted to each plot, the one on the outside of the bed next to the board being discarded. There were two plots to each row and 144 rows, so that the whole area taken up by the experiment was 448 square feet or about one hundredth of an acre.

THE PLAN.

To have distributed 288 plots all planted at different times in straightforward randomized blocks would have been a virtual impossibility, the practical difficulty of planting many very small plots at different times a year being a very real one. It was thus essential to try some modification of the method which would make planting easier.

PLUM STOCK ROOT CUTTING TRIAL, 1931-2.

FIG. 1. ARRANGEMENT OF PRIMARY PLOTS.

Each Plot contains sixty cuttings.

KEY TO TREATMENTS :—

Time of Making Cuttings :

October 1931 (O)
December ,, (D)
January 1932 (J)
February ,, (F)
March ,, (M)
April ,, (N)

Variety :

Pershore (P)
Common Mussel (C)

Time of Planting :

At once (X)
Bedded in Sand until April (Y)

NOTE :—

(i) April-made cuttings replace those planned to be taken in November.

(ii) March cuttings discarded owing to error.

(iii) Block B is actually situated due north of Block A.

Block A.	Block B.
FCX	FPX
OPX	DPY
JPX	NCY
FPY	NPX
DCX	MCX
FPX	OPY
DPY	OCY
JCX	MCY
MPX	NPY
NCY	MPX
MPY	MPY
FCY	NCX
OPY	JPX
OCX	DPX
DCY	DCY
JPY	OPX
NPX	JPY
MCY	DCX
NPY	OCX
NCX	JCX
JCY	FCX
DPX	FCY
OCY	JCY
MCX	FPY

The experiment was therefore divided into two parts for the purpose of planning. The twenty-four combinations of variety, time of making, and time of planting, were designated primary treatments and were distributed in two randomized blocks (see plan, Fig. 1). These forty-eight primary plots each consisted of sixty cuttings and were subdivided into six secondary plots of ten cuttings each. The six different sizes of cutting were distributed at random within the primary plots (see plan, Fig. 2). The fact that each of these primary plots contained cuttings all of which were planted at the same time greatly lessened the risk of error in planting.

PLUM STOCK ROOT CUTTING TRIAL, 1931-2.

FIG. 2. ARRANGEMENT OF SECONDARY PLOTS.

Each secondary plot contains ten cuttings.

KEY :—
Size of cutting :
Length :
L = 12 cms. long.
S = 6 cms. ,,

Diameter :
1 = 3-6 mms.
2 = 6-9 mms.
3 = 9-12 mms.

S 3	L 1
S 2 FC X	L 3
L 2	S 1
L 1	S 1
S 2 OP X	L 3
S 3	L 2

III. PLANTING AND RECORDING.

During the procedure of making and planting the cuttings a record was kept of observations having a bearing on the practicability of the root cutting method of propagation. As these observations are not susceptible of statistical analysis it has been considered advisable to incorporate them in this section along with the outline of methods of planting and recording, leaving the tabulated data regarding survival and growth for presentation under a separate head entitled " Analysis of the Results ".

The soil in the cutting bed was of a sandy loam nature, friable and well drained. The entire bed was forked over and fined with a rake before setting the corner stakes for the primary plots. Each of these primary plots was re-dug and raked immediately prior to planting the cuttings.

The material from which the cuttings were made was secured by digging up three-year-old plants which had been cut to the ground each year. Roots of the two varieties, Pershore Egg and Common Mussel, were secured from the same plantation where they had received identical treatment up to the time of digging. A fresh lot of plants was dug at each time of making the cuttings. The digging was done by two workmen using strong forks. Special care was necessary to avoid damaging the roots, with the result that only about twenty whole roots per hour were lifted. Immediately after being dug, the roots were cut from the plants and wrapped in damp sacks.

Preparation of the cuttings was undertaken in a cool room the day after they were dug. A record was kept of the number of cuttings of each size grade secured from the roots of each plant. The average number of long stout cuttings, which subsequently proved to be the most satisfactory size, was about twelve per plant from the Common Mussel and six from the Pershore Egg. On account of the cost of growing and digging, this number of cuttings would hardly justify the growing of plants specially for the purpose of providing roots from which to make cuttings. In this connection, it may be well to mention, however, that in Europe, where two- and three-year-old trees are in demand, nurserymen have a source of propagating material in surplus roots pruned from the trees prior to sale. Even so, under most conditions, material for stem cuttings would be more readily obtainable.

In making the cuttings, the upper end was cut square across with a sharp knife, a slanting cut being made at the lower end to facilitate planting right end up. Difficulty was experienced in keeping the knife sharp owing to frequent turning of the edge by particles of grit adhering to the roots. Here again preparation of root cuttings involves more trouble than the preparation of stem cuttings.

As soon as the cuttings were made they were tied in bundles of the required number for planting in each plot, labelled and again wrapped in damp sacking. Small zinc labels were employed and these were attached to a wire stem about 1 foot long, to facilitate use of the original label in marking the final location of the cuttings in the planting bed.

In the actual operation of planting, a spacing board was used. This board consisted of a strip of 1 × 4, the width of the cutting bed, 55 inches, long. Eight-inch pegs were nailed along this board at $2\frac{1}{2}$ inch intervals. By means of this spacing board the location for each cutting was very quickly and accurately marked out.

Planting was done with a dibber, the holes being made deep enough so that at the completion of the planting operation, the top of the cutting was half an inch below the surface of the soil. Care was taken to firm the soil closely about each cutting.

Notes made regarding the condition of the soil at the time each batch of cuttings was planted revealed that soil conditions were most satisfactory in October, March and April. During the winter months, the soil was often very wet with the result that it was necessary to alter the planting schedule to avoid planting the cuttings at a time when soil conditions were unfavourable. These observations suggest the advisability of autumn or early spring planting of root cuttings.

Some of the surplus cuttings were bedded in sand and others were planted in a vacant space at one end of the planting bed at each planting date to provide a source of material which could be examined periodically without disturbing the cuttings actually included in the trial. Examination of this material on January 22nd indicated that cuttings of Common Mussel made on October 30th and bedded in sand had already developed shoots up to 1 inch in length but had as yet made very little root growth. In the permanent earth bed the shoots were shorter but some root growth had taken place. There was very little growth evident in the Pershore Egg cuttings either in sand or earth bed.

Again in April, when the bedded cuttings were set out, it was noted that the large and medium diameter cuttings of both Common Mussel and Pershore Egg prepared in October and December had made leaf shoots over 1 inch long. Some of the Common Mussel had made roots also, but very few of the Pershore Egg showed any sign of rooting. Hardly any of the small diameter cuttings of either Common Mussel or Pershore Egg showed any growth. The large and medium diameter January and February cuttings of both varieties showed some shoot growth, but practically no signs of root development. It was noted that Common Mussel cuttings tended to develop shoots all along their length, whereas in the Pershore Egg shoots were not so plentiful and developed near the top end of the cutting only. The percentage of cuttings showing shoot and root development was much smaller with Pershore Egg than was the case with Common Mussel. With both varieties more of the long cuttings showed root growth than was the case with the short cuttings.

From the above data it is apparent that differences in the behaviour of the various size grades of both varieties were evident very early in the growing season.

The plantation was kept weeded and cultivated throughout the growing period. A comparison of growth records obtained early in the Summer with the final survival figures obtained at the close of the growing season indicates that a number of cuttings, especially of the Pershore Egg variety, made several inches of stem growth but failed to become established and finally perished.

In spite of the involved layout of this experiment, the planting and recording presented no serious practical difficulties. A planting error which occurred in the March batch of cuttings, was due to mixture of the root cutting material received from the plantation and was not in any way caused by the complexity of the planting layout. In this connection it may be pertinent to remark that most fruit tree stocks are more difficult to identify from root characters than from stem characteristics. Thus, one disadvantage of the root cutting method of propagation lies in the difficulty of ensuring that the stock is kept true to variety.

TABLE I.

Actual Number of Plants Alive—October 1932.

In Two Plots of ten Cuttings each.

Common Mussel Plum.

Cuttings made in	October.				December.				January.				
Cuttings planted	At once.		In Spring.		At once.		In Spring.		At once.		In Spring.		A
Length	Long.	Short.	Long.	Short.	Long.	Short.	Long.	Short.	Long.	Short.	Long.	Short.	Lon
Diameter :—													
3-6 mms. . .	6	4	2	0	5	4	1	0	13	7	1	0	7
6-9 mms. . .	14	10	6	2	16	10	7	2	15	8	4	3	13
9-12 mms. . .	18	11	14	3	17	10	15	10	18	8	10	2	14
Totals for each Length . .	38	25	22	5	38	24	23	12	46	23	15	5	34
Totals for each Time of Planting	63		27		62		35		69		20		
Totals for each Time of Making	90				97				89				

* These plots were planted on the same date.

A layout such as that used in this experiment facilitates accurate interpretation of results by observational methods. Thus, in the experiment under discussion, many of the conclusions were evident to a close observer even before the survival and growth data had been tabulated and submitted to statistical analysis.

IV. ANALYSIS OF THE RESULTS.

The success or otherwise of the methods used in this experiment is shown in two ways. Firstly, the proportion of cuttings which actually took root and grew must be considered. This was measured by the number which were still alive in October 1932. Secondly, the growth made during the first season is of importance, since, though a cutting may be alive and rooted, it may have to be grown on for another season before it can be used as a rootstock. On the other hand, a proportion of the cuttings may make sufficient growth for use as rootstocks straight away. The growth, however, is not very easy to measure. It might seem that the average height of the plants at the end of the first season would have shown all that was necessary. Unfortunately, however, it was not possible to place any great reliance on this record. The number of cuttings which remained alive varied from nought to ten plants per plot, i.e. from complete failure to a full plant. Where there were only one or two cuttings in a plot, these often made good growth largely because they had plenty of room; whereas it seems probable that where a full plant existed, the plants suffered to some extent from overcrowding. The total amount of growth per plot of ten cuttings was therefore taken as the best measure, which would combine both survival and growth in a single record; and this record considered in the light of the earlier survival record gave, as far as the plots having comparable numbers of living plants were concerned, a very fair indication of their relative growth.*

The first section of the analysis, therefore, deals with the actual number of plants alive in October 1932.

(a) NUMBER OF PLANTS ALIVE.

The first result is easily told and considerably simplifies the issue, though the accuracy of experiment was decreased thereby. The variety Pershore Egg, which is well known as difficult of vegetative multiplication, was to all intents and purposes a complete failure. The percentage " take " of the root cuttings was so small that no degree of success could be attributed to any of the treatments practised and in consequence this variety will not be further considered.

* Since these data were analysed, the advantages of the " Analysis of Covariance " method of examining this kind of data have come to be more fully realized. Though its use here is unlikely to bring out any further information, it probably provides a simpler statistical technique for the analysis of experiments where the growth of the plants is partly dependent upon the number which survive.

Nevertheless, it may be noted that a few stray cuttings did take root and made good plants ; so that although these were unconnected with any particular treatment, it seems possible that further research may result in finding a method of growing this variety from root cuttings.

Owing to an error in planting, the whole of the March batch of cuttings had further to be ignored—the wrong variety being planted in a proportion of the plots.

The analysis is, therefore, confined to the variety Common Mussel, cuttings of which were made in October, December, January, February and April.

COMMON MUSSEL PLUM. ROOT CUTTING TRIALS, 1931-2.

NUMBER OF PLANTS ALIVE PER PLOT OF TEN CUTTINGS, OCTOBER 1932.

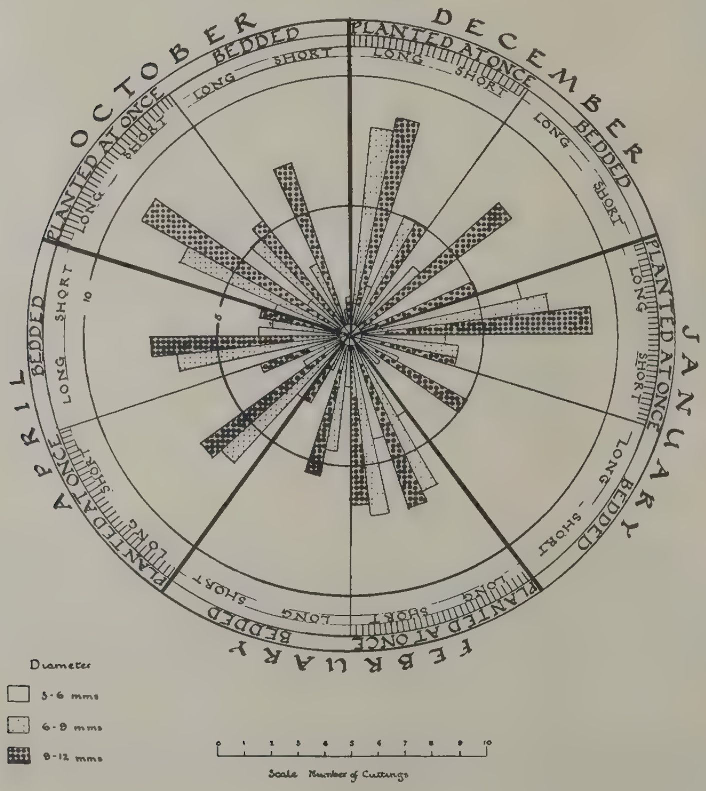

FIG. 3.

Table I shows the actual numbers of cuttings which remained alive out of a possible twenty for each treatment. Thus, there were 240 cuttings made in October 1931, ninety of which were alive a year later. Of these ninety plants, sixty-three were from cuttings planted at once, and twenty-seven from cuttings bedded and planted out in Spring. The numbers for each size grade are shown in the body of the table.

These data are illustrated in Fig. 3. In this diagram a circle is divided up into sixty sectors, one for each combination of treatment and size. The *radius* of each sector represents the relative number of plants alive for the combination. The circle is first divided into five sectors representing the five different months. These are subdivided into two for the different plantings. Each of these subdivisions contains six sectors each representing one of the six different grades of cutting. Thus, reading in a clockwise direction, can be seen October, Planted at once, Long cuttings, Diameter 1, 2 and 3 ; October, Planted at once, Short cuttings, Diameter 1, 2 and 3 ; October, Bedded, Long cuttings, Diameter 1, 2 and 3 ; October, Bedded, Short cuttings, Diameter 1, 2 and 3 ; and so on.

The analysis of these data is somewhat different from the ordinary analysis of variance as applied to the yields of field experiments. In this case the results are taken from a discontinuous distribution, that is to say, they are in the form of whole numbers varying from nought to twenty for each combination of treatment and size.

The simplest way of testing the significance of these results is, therefore, to construct a series of what are known as " Contingency Tables " for each issue and combination of issues in the experiment.

For example, the question of planting the cuttings at once as against the practice of bedding and planting in Spring may be considered. Table II shows that out of a possible 1,200 plants, combining all treatments, 481 survived and 719 died.

TABLE II.

Time of Planting.

	At once.	In Spring.	Total.
Alive	309	172	481
Dead	291	428	719
Total	600	600	1,200

Of these 1,200, 600 were planted at once—of which 309 survived and 291 failed, and 600 were bedded till Spring, of which only 172 survived and 428 died.

Now if time of planting had made no difference to the survival, and both batches had behaved exactly alike, there would have been 240·5 alive and 359·5 dead in each case, these expected figures being directly proportional to the marginal totals. Do the observed figures differ significantly from this expectation ?

The significance of a departure from expectation is tested by calculating the statistic known as χ^2 which measures the discrepancy between observation and hypothesis. This is known as a test of " Goodness of Fit " and χ^2 is calculated from the formula

$$\chi^2 = \sum \frac{x^2}{m}$$

Where x is the difference between the expected value and the observed value and m the expected value for each " cell " of the table. The sum of the contributions from each cell is the required figure for the whole table.

In the present table the differences between observation and hypothesis are the same in all four cases, viz. 68·5, so that

$$\chi^2 = 2\left\{\frac{68{\cdot}5^2}{240{\cdot}5} + \frac{68{\cdot}5^2}{359{\cdot}5}\right\} = 65{\cdot}125.$$

This value must be compared with the value which can normally occur by chance. A table showing the probability of occurrence (P) of values of χ^2 for different values of " n " the number of degrees of freedom, is given by Fisher (1). This table must be entered with " n " equal to the number of degrees of freedom in the contingency table. In the present instance there are four cells to the table, but, knowing the marginal totals, if one of these cells is fixed the other three must fill themselves automatically and there is thus only one degree of freedom.

Examining the table of χ^2, it is found that a value of 6·63 will occur normally once in 100 times (i.e. the Probability $P = {\cdot}01$). The value found of 65·125 is very much greater than this ; so that there is no possible doubt that the divergence is significant.

This fact was, of course, obvious, but the analysis is carried through in detail here as an example to show how the various issues were tested.

Taking next the issue of time of making cuttings, the totals in the bottom row of Table I show the number alive for each month. Actually there is somewhat less variation between these totals than would normally be expected and it is certain that no real difference between them exists.

Considering length of cutting, Table III shows that, as in the case of time of planting, the significance of the deviation from expectation is unquestionable, the expected values in this case being the same as in Table II, and there is no doubt that long cuttings have given better results than short. Similarly in the case of diameter, it is evident that the stouter the cutting the better the " take "

TABLE III.

Length of Cutting.

	Long.	Short.	Total.
Alive	310	171	481
Dead	290	429	719
Total	600	600	1,200

(Table IV), the expected values for each diameter being 160·3 alive and 239·7 dead, though there is not such a great difference between the two larger grades as between the two smaller.

Summing up the straightforward results of single issues; there were an approximately equal number of plants alive from cuttings taken in each of the five months, but very many more plants resulted where the cuttings were planted at once than where planting was delayed till Spring. Long cuttings were definitely better than short, and stout cuttings proved better than thin; all these points are obvious in Fig. 3.

TABLE IV.

Diameter of Cutting.

	3-6 mms.	6-9 mms.	9-12 mms.	Total.
Alive	88	170	223	481
Dead	312	230	177	719
Total	400	400	400	1,200

It now becomes necessary to examine the interactions of two or more issues. An obvious example is the interaction of time of planting and time of making (see last line but one of Table I); for though there are big differences between the two groups, planted at once, and in Spring, in the October, December, January and February plots, naturally there is no real difference between them in April, since both lots were planted at the same time. But both these lots had somewhat fewer cuttings alive than those taken and planted at once in the other four months. The significance of these interactions is tested as before, but now the tables are slightly more complex.

As an example, the interaction of time of planting and length of cutting is the simplest. Ignoring April, since in this month all were planted together, Table V shows the observed numbers alive and dead for each of the four combinations of time of planting and length, there being a possible 240 in each

TABLE V.

Interaction of Time of Planting and Length of Cutting.

Length of Cutting.	Alive.			Dead.		
	Time of Planting.		Total.	Time of Planting.		Total.
	At once.	In Spring.		At once.	In Spring.	
Long	156 (*167*)	84 (*73*)	240	84 (*73*)	156 (*167*)	240
Short	107 (*96*)	31 (*42*)	138	133 (*144*)	209 (*198*)	342
Total	263	115	378	217	365	582

case, e.g. out of 240 long cuttings planted at once there were 156 alive and eighty-four dead. Alongside each of the observed numbers is shown (in italics) the expected number, if each combination had acted in proportion to the marginal totals. Thus if the 240 long cuttings and 138 short cuttings which remained alive had behaved the same whether planted at once or in Spring they would have occurred in the same ratio, i.e. 263-115 in each case.

Comparing observed and expected, χ^2 is found to be 10·329, a value which will be exceeded by chance somewhat less than once in 100 times. Thus, here again there is a significant divergence showing that the relative mortality of the short cuttings was higher when they were bedded in till Spring than when planted at once.

It is not proposed to describe the process of analysis in detail further; the principle is exactly the same when the second and third order interactions are considered; but the process becomes somewhat more laborious and it is sufficient to indicate the significant results. These may be summarized as follows:

1. There was no significant difference in the total number alive from the plantings made from cuttings taken in different months, but there was a slightly higher mortality in April, if those planted at once only are compared.
2. Bedding in sand was detrimental to all the four groups which were subjected to it.
3. Size of cutting was most important, the long stout cuttings being always more successful than the short thin ones.
4. The short thin cuttings suffered more from the bedding in sand than the long stout ones.
5. This was especially the case in the January and February groups.
6. In April there was less difference due to diameter than in other months especially in the case of the short cuttings.

(b) GROWTH OF CUTTINGS.

Having discussed the number of plants which resulted from these root cuttings, the second point to consider is the comparative growth of the plants which survived during their first season. As has already been explained, this may be determined by comparing the total recorded growth per plot with the number of plants in those plots ; for, owing to the spacing factor, the growth in plots with few plants cannot fairly be compared with that in plots having a comparatively full plant.

It is considered advisable, therefore, to analyse the growth record as a whole and then reconsider the results in the light of the survival factor.

It has been shown in the description of the layout of the trial that the experiment was arranged in two parts. Firstly, the ground was divided into plots of sixty cuttings, in which the combinations of variety, time of making and time of planting were distributed ; secondly, the plots were subdivided into six sub-plots, in which were distributed the six different sizes of cutting.

Since this record is a record of growth, and the data can take any values from nought upwards, the analysis can be done in the ordinary way using the analysis of variance method. This method is too well known to necessitate explanation of the principles in this paper, but the uninstructed reader may be referred to the numerous papers by Fisher and others in which the application of the analysis of variance to field experimental data is described (1, 2, 3).

In this particular case the experiment is a somewhat complex one, and the analysis is perhaps rather more difficult. As the layout is in two parts, so there are two sections to the analysis. First, the analysis is carried out for plots of sixty cuttings and (since one variety is eliminated) is simply that for two randomized blocks each containing ten plots.

The second part of the analysis is that of plots of ten cuttings and may be regarded primarily as the analysis of an experiment consisting of twenty randomized blocks, each containing 6 plots for the six size differences. A large part of the variance usually attributed to the errors of the experiment, is in this case however, known to be made up of the variation due to the interactions of size of cutting with times of making and planting. Thus, this part of the variance has to be further split up in order to examine the significance of these interactions. The full analysis of a complex experiment has been described by Wishart (8). In this paper the results only will be considered.

The average growth per plot of ten cuttings for each combination is shown in the body of Table VI and further illustrated in Fig. 4. This diagram is the same in form as Fig. 3, the only difference being that the length of the radius of each sector now represents average growth per plot instead of number of plants.

The first part of the analysis deals with plots of sixty. The average growth per plot of sixty cuttings for each combination of time of making and time of planting is given in the last line but one of Table IV, and the average growth per time of making in the bottom line, it being remembered that the growth for April is the mean of two batches both planted at once.

COMMON MUSSEL PLUM: ROOT CUTTING TRIALS, 1931-2.

TOTAL GROWTH IN CENTIMETRES PER PLOT OF TEN CUTTINGS,
OCTOBER 1932.

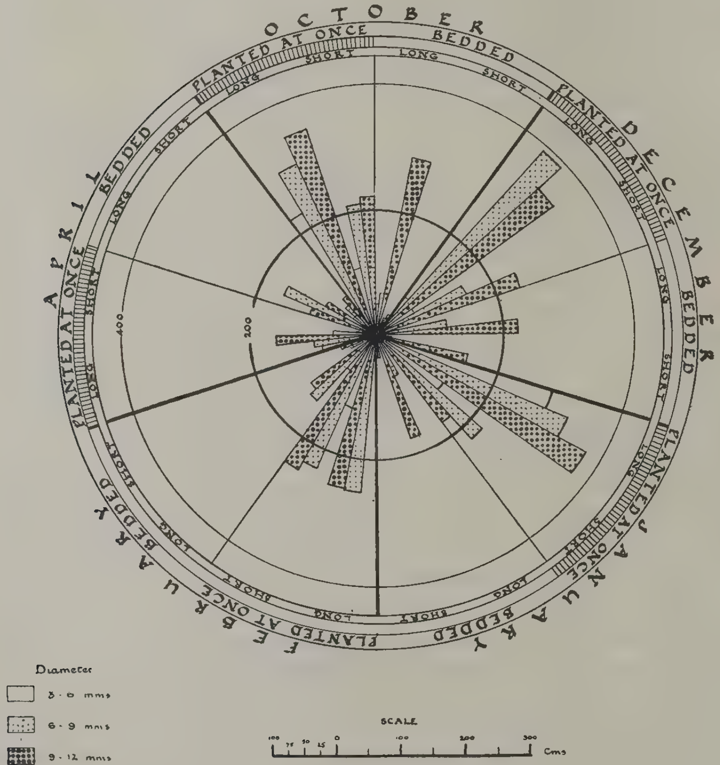

FIG. 4.

Considering the first four months, it is of course obvious that there is an enormous difference in total growth between the plots planted at once and those bedded until Spring, but that there is no significant difference between the four months. The growth of the April cuttings on the other hand was very much less

TABLE VI.

Average Growth in Centimetres—October 193

Per Plot of Ten Cuttings.

Common Mussel Plum.

Time of Making	October.				December.				January.				
Time of Planting	At once.		In Spring.		At once.		In Spring.		At once.		In Spring.		At
Length of cutting	Long.	Short.	Long.	Short.	Long.	Short.	Long.	Short.	Long.	Short.	Long.	Short.	Long
Diameter :—													
3-6 mms. ..	228	108	44·5	0	76·5	66	12	0	287·5	130	7	0	158·
6-9 mms. ..	303·5	210·5	64·5	29	391·5	154·5	111·5	22·5	325	224·5	70	38·5	253·
9-12 mms. ..	347	221·5	288·5	34·5	341·5	238·5	222·5	145	374	173·5	192	17·5	252
Mean for each Time of Planting	1418·5		461		1268·5		513·5		1,514·5		325		1,2
Mean for each Time of Making	939·75				891				919·75				

than that of comparable plots. The standard error of the mean of two plots is 138·6 cms. or 16·9 per cent. of the general mean, which is rather high, and the difference which is significant is 443·4 cms.

The variation between like plots was rather higher than was expected (the coefficient of variability was 23·9 per cent.). If the second variety had survived, the standard error for the comparison of the means of the different treatments would have been lower since it would be based on the growth of double the number of plants. Nevertheless the main differences are quite definite.

Examining these figures in relation to the number per plot, the difference between those planted at once and in Spring is to be expected, because of the lower number of plants. Nevertheless taking into consideration the crowding factor, which probably had some effect on the plots with a nearly full plant, and the fact that there were only about twice the number alive in the plots planted at once, whereas they have made about three times the amount of growth, there are some grounds for supposing that bedding until Spring had some detrimental effect on growth as well as survival. On the other hand, there is no possible doubt that cuttings made and planted in April, although they had nearly as high a rate of survival as the other months where the cuttings were planted at once, made very much less growth.

These facts lead to the conclusion that the poor result in the bedded plants was largely due to some factor in the bedding of the plants causing a high mortality, rather than to the delay in planting, since these cuttings behaved quite differently to the April batch which survived but made very poor growth.

The reason for this is not readily apparent. An analysis of sand was obtained and is given in the Appendix, but there seems no reason for the sand itself to have been detrimental.

The frames in which the plants were bedded were kept open and being exposed to the South West are the wettest frames on the Station. In addition they were kept watered, so that dryness can hardly have been a serious factor.

Turning now to the second part of the analysis, i.e. that of plots of ten cuttings, it is evident from Table VII that size of cutting made a great deal of difference to the total growth. The variation coefficient was 39·2 for small plots and the standard error of the means in Table VII, each figure of which is the average of twenty plots, is 12·0, showing a significant difference of 34·0 cms. per plot.

These differences are probably entirely due to the numbers which survived. The average number alive is shown in italics under the growth figures and it is noticeable that the growth is very nearly proportional to the number of plants.

The relative importance of length and diameter is also shown in this table; and seems to indicate that length may possibly be of more importance than

Table VII.

Mean Growth in Centimetres per Plot of Ten Cuttings.

Length.	Diameter.		
	3-6 mms.	6-9 mms.	9-12 mms.
Long (12 cms.)	108 *(2·7)*	191 *(5·5)*	240 *(7·3)*
Short (6 cms.)	53 *(1·7)*	105 *(3·0)*	124 *(3·8)*

General Mean = 136·9. S.E. = 12·0. Sig. Diff. = 34·0.

diameter. The success of the cuttings seems to be directly proportional to their length. As far as diameter is concerned, though there is a very big difference between the two smaller grades, increasing the diameter further does not give a corresponding increase in growth, especially in the case of the short cuttings.

This is not, however, invariably the case as is shown in Table VIII, for though there is little difference between the two larger diameter grades in the January and February lots and very little due to diameter at all in the April lots, there are considerable differences between all three grades in the October and December lots.

Table VIII.

Interaction of Time of Making Cuttings and Diameter.

Mean Growth per Plot of Ten Cuttings, and Number of Live Plants.

Diameter.	Time of Making.				
	October.	December.	January.	February.	April.
3-6 mms. ..	95·1 *(1·5)*	38·6 *(1·2)*	106·1 *(2·6)*	88·2 *(2·4)*	75·6 *(3·2)*
6-9 mms. ..	151·9 *(4·0)*	170·0 *(4·4)*	164·5 *(3·7)*	167·4 *(4·9)*	87·0 *(5·5)*
9-12 mms. ..	222·9 *(5·7)*	236·9 *(6·5)*	189·2 *(4·7)*	170·9 *(5·5)*	89·5 *(5·4)*

General Mean = 136·9 cms. S.E. = 19·0. Sig. Diff. = 53·7 cms.

This result has been partially forecast in the preliminary analysis of the number of plants alive and is shown to be dependent upon this factor by the average numbers per plot given in italics in Table VIII.

An examination of Table VI, however, seems to indicate that the superiority of the thickest cuttings in the early months is chiefly to be found in those which were bedded. It is possible, therefore, that the greater thickness enabled them to survive this treatment better.

V. GENERAL CONCLUSIONS.

Summarizing the results of this experiment, the dramatic difference in response of the two varieties drives home the lesson that it is dangerous to draw general conclusions from an experiment on a single variety. Secondly, the detrimental effect of bedding in sand under these conditions was hardly less emphatically shown by the high mortality among these cuttings, though whether the subsequent growth of those which survived was affected or not is less clear. April was obviously too late a time to take cuttings, for though the survival was fair, the growth was very poor. Figs. 5 and 6 show the difference between the April plants and those from cuttings made and planted in October. There was apparently little difference between the cuttings taken and planted in the other months, but this factor needs further trial, since the Winter was, on the whole, a mild one and in other seasons the results might be very different.

Diagrams showing the weather conditions have been prepared but are omitted here from lack of space. It may be noted, however, that there is no obvious connection between either rainfall or temperature with the results obtained.

Size of cutting had a marked effect on survival. It was evident that cuttings less than 6 mms. in diameter were of little value and that those 12 cms. in length were much more likely to grow than the short cuttings. Those which survived, however, grew as well as the long cuttings, though it is questionable whether this would have been the case had the cuttings been spaced a little farther apart. It is possible, therefore, that where material is limited, it might be advisable to decrease the length somewhat (say, to 9 cms.), for the larger number of cuttings available might compensate for the greater mortality, since in the present case the number of short (6 cm.) cuttings, which rooted and grew was rather more than half the number of plants which were obtained from cuttings twice their length.

The success which attended the planting of Common Mussel cuttings of desirable size at a suitable time is evidence that the root cutting method of propagation provides a practical means of increasing the supply of at least one variety of rootstock. Nevertheless the observations recorded during the preparation of the cuttings indicate that this method of propagation has several disadvantages which are likely to prevent it from coming into general use with stocks which can be readily propagated by stem cuttings or layerage.

Finally, the information secured regarding the inter-relation of the various factors involved in the successful production of stocks from root cuttings is ample justification for the somewhat complex labour of the plots and the involved methods of analysing the results.

Although the differential responses obtained were here of rather minor importance and may seem hardly to warrant the elaborate statistical methods applied to the data, this is by no means a likely result in most experiments of this kind. This trial was primarily an essay in technique and as such marks a new departure in this class of horticultural experiment. In view of the world-wide attention which is now being paid to methods of vegetative propagation and the consequent need for experimental work in this direction, it was thought worth while to describe in full not only the layout and field technique but also to indicate the statistical methods which may be applied to results of this kind.

ACKNOWLEDGMENTS.

Acknowledgments are due to F. W. Arnaud, Esq., who analysed the sand ; to Professor R. A. Fisher, who gave valuable advice in the analysis of the survival figures ; to R. G. Garner, Esq., who recorded the growth data ; and to Miss B. Carter, who drew the diagrams.

SUMMARY.

1. A complex experiment, including several factors relative to the propagation of plum rootstocks from root cuttings is described ; the advantages of this type of experiment are discussed.

2. The layout of the experiment is described.

3. The methods of making and treating the cuttings, and recording their survival and subsequent growth are included and the practical disadvantages of this method of propagation, as compared with that of stem cuttings in a variety that is easily propagated in this way, are pointed out.

4. The analyses of the numbers which were alive at the end of the first season and the growth which they had made are described and the results enumerated.

5. Whereas the variety Pershore Egg did not respond to this method of propagation, a considerable degree of success was attained with Common Mussel.

6. In this rather mild winter, with the exception of those planted in April, there was little difference in survival or growth of those cuttings made and planted in October, December, January and February. Those made and planted in April had a somewhat lower survival and made very poor growth.

7. Bedding in sand until the Spring was, under the conditions of this experiment, always detrimental.

8. Size of cutting had a considerable influence on the survival, both length and diameter being of importance. Cuttings 9 inches in length and not less than 6 mms. in diameter would seem on the whole likely to give the best results.

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APPENDIX.

Analyses of Sand Used for Bedding the Root Cuttings.

	per cent.
Water	0·065
Siliceous matter insoluble in hydrochloric acid	99·40
Oxides of iron and alumina	0·23
Lime (CaO)	0·04
Organic matter (loss on ignition)	0·12
	99·855
Ferrous iron	0·01
Arsenic	Absent

The only apparent impurity in the sand was a little clinker.

The mechanical analysis was as follows :

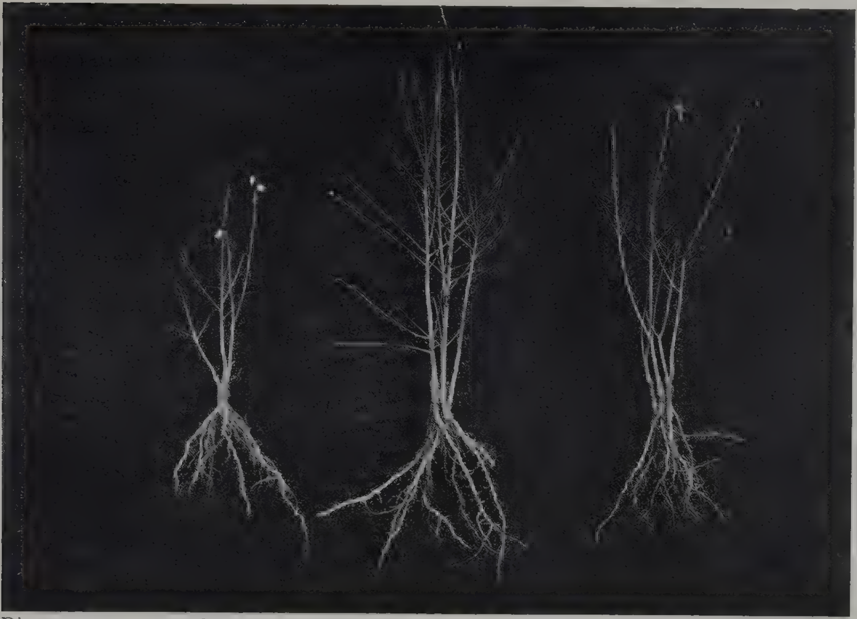
Coarse sand	94·5	per cent.	(residue on 100 Sieve).
Fine sand	3·0	,, ,,	(between 0·2 and 0·04 mm.).
Silt and fine silt ..	0·4	,, ,,	(between 0·04 and 0·002 mm.).
Clay	none		
	97·9		

(*Received* 27/10/33.)

Fig. 5. October Planting—Common Mussel Plum.

Rootstocks grown from a plot of ten root cuttings made and planted in October 1931 and lifted in October 1932.

Short Root Cuttings.


Diameter
3-6 mms.
6-9 mms.
9-12 mms.

Long Root Cuttings.

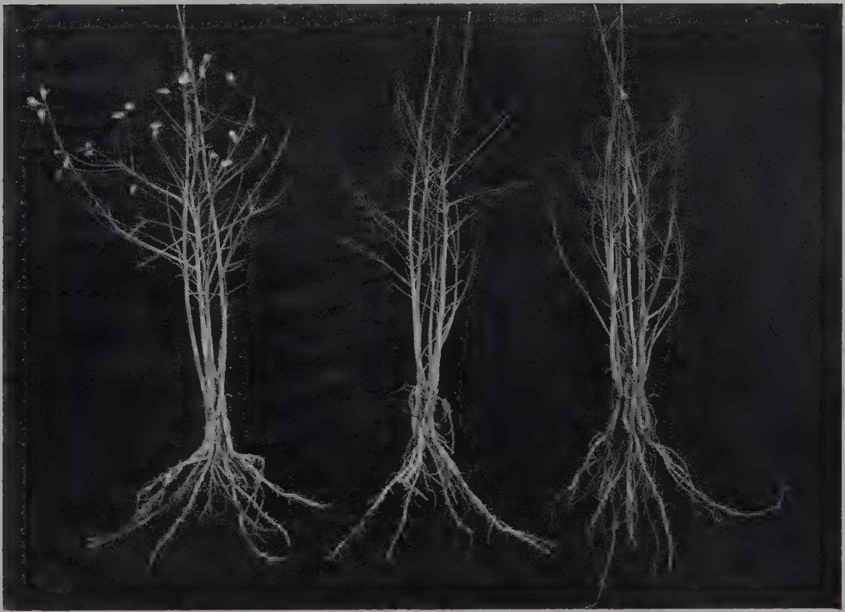
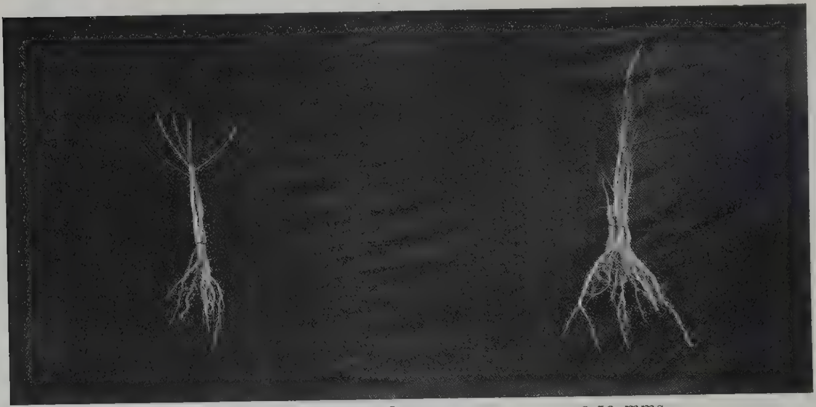

Diameter
3-6 mms.
6-9 mms.
9-12 mms.

Fig. 6. April Planting—Common Mussel Plum.

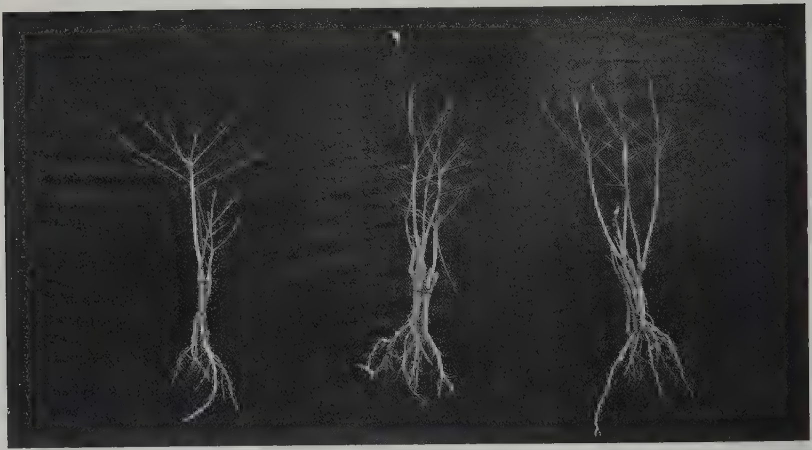
Rootstocks grown from a plot of ten root cuttings made and planted in April 1932 and lifted in October 1932.

Short Root Cuttings.



Diameter 3-6 mms. 6-9 mms. 9-12 mms.

Long Root Cuttings.



Diameter 3-6 mms. 6-9 mms. 9-12 mms.

SPRAYING AND DUSTING EXPERIMENTS ON THE CONTROL OF APPLE SCAB *(VENTURIA INAEQUALIS)* AND APPLE MILDEW *(PODOSPHAERA LEUCOTRICHA)* AT EAST MALLING IN 1931-1932

By **M. H. MOORE**
East Malling Research Station

INTRODUCTION.

GENERAL.

THESE field trials, carried out in 1931 and 1932, were the outcome of similar tests made in previous years. It was felt that the original experiments had served their purpose by the end of 1930, and thus it was stated in a previous publication (7) that " after 1930, the experiments were re-planned in order to develop other aspects of the problem which had arisen as the work proceeded ". Much of the experience and knowledge gained in the early trials—fully described in (3) and (7)—was embodied in the experiments herein discussed, and advantage was taken of the opportunity to probe a vexed question under English conditions ; that of the efficacy of dusting compared with spraying for Scab- and Mildew-control. A brief preliminary paper (5) dealing with certain aspects of these re-planned trials has already appeared, although when it went to press complete data had not been obtained. The current paper is intended to present and discuss in full the complete data obtained during the two years' work which is now terminated.

THE TREES.

The trees used were Cox's Orange Pippin planted as " maidens " in 1921. They were " worked " on various layered rootstocks, and, in general, there were sixteen trees of each stock-scion combination. All previous treatment (such as pruning, manuring, cultivation) apart from spraying, had been as uniform as possible, but, in using the same trees for the new trials as for the old, it was inevitable, and, indeed, very desirable, that certain " controls " in the old trials should be " treated " in the new, and vice versa. It could thus be determined whether a tree, unsprayed for years, could be brought back into good condition (freedom from pest and disease) by one season's thorough treatment.

THE FUNGICIDES.

So much damage had been caused on Cox's in previous years by " full-strength " Bordeaux mixture (8-8-100 with burnt lime, or 8-12-100 with hydrated lime) that it was resolved to restrict the use of this spray to a minimum. A few trees served to test a half-strength mixture made to the arbitrary formula 4 lb. copper sulphate, 18 lb. hydrated lime, 100 gallons water, and used pre- and post-blossom, but most of the treated trees received sulphur preparations as sprays or dusts. The sprays used were commercial lime-sulphur (specific gravity 1·3, polysulphide sulphur 25 per cent. weight in volume) and colloidal sulphur (40 per cent. sulphur by weight) ; sulphur dust was made up by a commercial firm to a specification supplied by the writer as follows : by weight, 95 per cent. sulphur, 5 per cent. china clay (to aid distribution of dust), 90 per cent. of the mixture to pass through a 300-mesh sieve.

All trees received lead arsenate as spray or dust at pink-bud and petal-fall (except those on No. XIII stock) to control caterpillars and other biting insects. Thus, on control trees, the arsenate was applied alone, but on trees treated with a fungicide, the arsenate was added to the fungicide. For sprayed trees, commercial lead arsenate paste was incorporated with lime-sulphur, while for dusted trees, lead arsenate powder (10 per cent.) was mixed up by the firm with the sulphur (85 per cent.) and china clay (5 per cent.). The controls in each series respectively received lead arsenate paste in water and lead arsenate dust (10 per cent.), in china clay (90 per cent.).

The sprays were applied at the following strengths :

Lime-sulphur, 1-30 pre-blossom, 1-100 post-blossom.
Colloidal sulphur, 8 lb. per 100 gall. pre-blossom, 4 lb. per 100 gall. post-blossom.
Lead arsenate paste, 4 lb. per 100 gall. pre- and post-blossom.
Spray gelatine (spreader and sticker), 1 lb. per 100 gall. pre- and post-blossom.

TIMES OF APPLICATION.

For the first time in this series of experiments a trial of two pre-blossom sprays against one was made. No evidence had been obtained that under East Malling conditions two pre-blossom sprays are essential to good Scab-control, although in other parts of the country, notably at Long Ashton in the West, different conditions may render two desirable (2).

The first application was made at the green-bud stage (see (6) Fig. III) and the second at the pink-bud stage. Post-blossom applications were made at petal-fall and three weeks later, while, on a few trees on No. VI stock, four post-blossom dusts were applied at ten-day intervals from petal-fall.

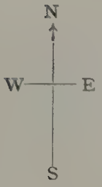
METHODS OF APPLICATION.

A mobile, horizontal-barrel machine, pumped by hand, delivered the sprays at a pressure of 200-220 lb. per square inch through a " spray-gun " lance adjusted to give a medium spray. Dusts were applied by means of a small dusting machine, operated by hand, illustrated in Fig. I.* Large hessian screens were used as before to prevent drift.

General Plan of Trials.

It would have been desirable in these re-planned experiments for all the trees to be worked on the same rootstock. In the earlier trials, much useful information had been acquired on the influence of rootstock on infection by Scab, for they had been planned with this end in view. In altering the arrangement, however, the presence of different rootstocks proved a handicap, which was overcome as far as possible by arranging that trees on each rootstock constituted a self-contained experiment. Thus, of sixteen trees, eight were treated and eight were controls; of eight trees, four were treated and four were controls.

Cox's O.P. on :



Tree										
16	VIt	It	Xc	XIIIc	XVIc	IIIt	IXc	IIt	Vt	IVt
15	VIc	Vc	IIc	IXt	IIIc	XVIt	XIIIt	Xc	Ic	XVc
14	VIt	Ic	Xt	XIIIt	XVIt	IIIc	IXt	IIc	Vc	IVc
13	VIc	Vt	IIt	IXc	IIIt	XVIc	XIIIc	Xt	It	XVt
12	VIt	It	Tree missing.	XIIIc	XVIc	IIIt	IXc	IIt	Vt	IVt
11	VIc	Vc	IIc	IXt	IIIc	XVIt	XIIIt	Xc	Ic	XVc
10	VIt	Ic	Xt	XIIIt	XVIt	IIIc	IXt	IIc	Vc	IVc
9	VIc	Vt	IIt	IXc	IIIt	XVIc	XIIIc	Xt	It	XVt
8	XVt	It	Xc	XIIIc	XVIc	IIIt	IXc	IIt	Vt	VIt
7	IVc	Vc	IIc	IXt	IIIc	XVIt	XIIIt	Xc	Ic	VIc
6	XVc	Ic	Xt	XIIIt	XVIt	IIIc	IXt	IIc	Vc	VIt
5	IVt	Vt	IIt	IXc	IIIt	XVIc	XIIIc	Xt	It	VIc
4	XVt	It	Xc	XIIIc	XVIc	IIIt	IXc	IIt	Vt	VIt
3	IVc	Vc	IIc	IXt	IIIc	XVIt	XIIIt	Xc	Ic	Tree missing.
2	XVc	Ic	Xt	XIIIt	XVIt	IIIc	IXt	IIc	Vc	VIt
1	IVt	Vt	IIt	IXc	IIIt	XVIc	XIIIc	Xt	It	VIc
Row	1	3	5	7	9	10	12	14	16	18

t—treated. c—control.

The alternate rows are occupied by Stirling Castle trees.

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* The writer is indebted to Mr. W. Steer, of the Entomology Section, for kindly taking the photograph.

It will be seen that the centre line divides the plot into two similar halves. Each half contained an equal number of treated and of control trees on every rootstock.

In previous experiments (3, 7) it was established that three applications of lime-sulphur, at pink-bud, petal-fall and three weeks later, provided an excellent basic spray-programme for good control of Scab, Mildew and Red Spider. Using this programme as a standard of comparison, therefore, the chief aim in the present trials was to estimate the relative values of various spraying and dusting schemes, the importance of the main three periods of application being taken for granted.

The trials were arranged so that comparisons of efficiency might conveniently be made between :

(a) dusting and spraying.
(b) lime-sulphur and colloidal sulphur.
(c) lime-sulphur and " half-strength " Bordeaux mixture.
(d) lime-sulphur with gelatine and without.
(e) two pre-blossom sprays and one.

In all cases of reference to fungicides, it will be assumed that the presence of lead arsenate is understood. It has already been shown that lead arsenate, although not an accredited fungicide, may have some influence on the toxic properties of a fungicide with which it is incorporated (1, 3, and 7).

METHODS OF RECORDING AND INTERPRETING RESULTS.

(a) *Apple Mildew.*—All infections had been cut out in the Spring of 1931, so that the trees were given a fresh start for the new experiments. As formerly (3, 7), mildew-infection was calculated as a percentage of total shoots for each tree. Infections were not cut out at the beginning of 1932 in order that cumulative effect of treatment might be studied.

(b) *Scab on Leaves.*—Two estimations were made in 1931, the first just before the second post-blossom application, and the second a few weeks afterwards. Estimations were made as in earlier experiments, the degree of Scab-infection on each tree being assessed numerically by general inspection, the convention 0 (free) to 5 (severely attacked) being used. In 1932, time was found for only one estimation, which was made a few days before the second post-blossom application.

(c) *Dropped Fruits.*—These were collected from petal-fall to picking-time, and all were graded for Scab and Sawfly-attack. The figures relating to Apple Fruit Sawfly have already formed the subject of a separate paper (10), so they will not be considered here. Scabbed fruits among the dropped ones were expressed as a percentage of the total number dropped.

(d) *Picked Fruits.*—These were graded for Scab-infection and russeting by the "category" method (fully described in previous papers (3, 9)). Every fruit was graded, for the total crop was scarcely of sufficient proportions to warrant the use of a sampling method. Examples of the methods used in calculating the Scab-Equivalents and the Relative Efficiencies* of the sprays have been published in detail in connection with Pear Scab (9), and therefore they will not be repeated here.

EXPERIMENTS IN 1931.

TIMES OF SPRAY- AND DUST-APPLICATIONS.

1.	Green-bud (see (6) Fig. III)	April 21st.
2.	Pink-bud	May 4th and 6th.
3.	Petal-fall	May 26th and 27th.
3a.	Ten days after 3	June 6th (dust only, on trees on No. VI stock).
4.	Three weeks after 3	June 16th.
4a.	Ten days after 4	June 26th (dust only, on trees on No. VI stock).

NOTES ON WEATHER CONDITIONS IN RELATION TO SPORE-DISPERSAL AND BUD-DEVELOPMENT.

The first ripe ascospore was found (in dead leaves kept under natural conditions in a wire cage) on April 29th, less than a week before the pink-bud application was made. Perithecia containing well-formed asci had been observed on these leaves more than a week previously, but the spores were not formed. The ascospores ripened rapidly during the first few days of May, and were being freely dispersed, as usual at East Malling, just before flowering time. Successional production of conidia from Scab-pustules on the wood of unsprayed trees normally occurs before ascospores are ripe and for some time afterwards, but no leaf-infection was found on any trees in these experiments before the pink-bud application was made.

It will be noted that the period from green-bud (April 21st) to petal-fall (May 26th) was about thirty-three days, and of this, the period from green-bud to pink-bud (May 4th) was twelve days, and from pink-bud to petal-fall, twenty-one days. A period of several days of very cold, rainy weather just prior to April 21st checked bud-movement, which, however, developed rapidly from then to pink-bud. General observation indicated that ascospore-development followed a similar course.

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* Efficiencies are calculated on the assumption that infection on the control trees represents the maximum (100 per cent.) for each separate stock-scion combination.

OBSERVATIONS ON EXPERIMENTAL AND COMMERCIAL DUSTING.

It must be emphasized that dusting trials were included in these experiments to discover, if possible, how sulphur dusts compared with sulphur sprays as agents in Scab-control ; it was not intended to show how commercial dusting should be carried out, for this would be premature.

It was soon evident that experimental dusting which involved the treatment of single trees planted amongst others treated differently, presented great difficulties that were not easily overcome. These difficulties have already been discussed in some detail in a previous paper (5).

In view of a general tendency to choose very still conditions for dusting, the first application (pink-bud) was made at 6.30 a.m. Although there was practically no wind, it was impossible, even by the use of very large hessian screens, to prevent the fine, light dust from drifting slowly in all directions. Furthermore, the trees were dripping with moisture, and the dust adhered to the leaves in patchy masses, each treated tree inevitably receiving much more dust than it was intended to have. When the moisture dried, the flakes of dust were easily blown away.

The next application (petal-fall) was made between 8 and 10 p.m. when there was little movement of the air and the trees were dry. The distribution of dust on the foliage was excellent, a fine film being deposited on both leaf-surfaces. The problem of drift, however, remained unsolved. It was impossible to prevent it under these conditions, and it was most undesirable that the dust should drift through sprayed and control trees.

In a final trial of methods (ten days after petal-fall), dusting was done on dry trees in the day-time while a light breeze was blowing. Although the operation was not perfectly satisfactory, it was a great advance on night and early-morning dusting, for the breeze blew the dust right through the tree, and into the large screens on the leeward side, where it was arrested. Excellent cover was obtained on both leaf-surfaces, even those most remote from the nozzle. A little drift escaped, but this was unavoidable. It was found that moisture falling on the dust-deposit was preferable to dust falling on wet leaves.

All points considered, however, the single-tree method is not advisable where experimental dusting is concerned. The loopholes for error and the difficulty of operation would off-set any statistical advantages gained by randomized distribution of single-tree units, and dusting trees in mass rather than singly is therefore desirable.

While these remarks apply to experimental dusting, it by no means follows that they are also applicable to commercial dusting. Still conditions and damp leaves might be much more suitable factors in commercial dusting where trees are treated in mass than they were in these experiments. This, however, is

TABLE I.

Control of Apple Scab and Apple Mildew, 1

Root-stock.	Treatment.		Treated or Control.	No. of Trees.	Mildew.	Leaves.				
	Pre-blossom.	Post-blossom.			Av. % Mildew per tree.	1 Av. Scab Equiv.‡	2 Rel. Efficiency† of Treatment.	3 Total No. Fruits Produced.	4 Total Scab-infected.	5 % Scab-infected.
I	LSA	LSA, LS.	T	8	5·6	0·25	94	433	40	9·2
	A	A, —.	C	8	10·5	4·50		194	87	44·8
II	LSA	S′A′, S′.	T	8	4·5	0·73	83	382	39	10·2
	A	A′, —.	C	8	11·0	4·25		346	151	43·6
III	CSA	CSA, CS.	T	8	4·5	0·98	70	430	47	10·9
	A	A, —.	C	8	6·6	3·25		248	98	39·4
IV	*BMA	*BMA, BM.	T	4	5·3	0·00	100	459	9	2·0
	A	A, —.	C	4	5·5	2·50		888	337	38·0
V	LSA, LSA	S′A′, S′.	T	8	5·6	0·45	89	529	62	11·7
	A, A	A′, —.	C	8	15·0	4·00		240	79	32·9
VI	S′A′	S′A′, S′, S′, S′.	T	8	2·9	1·34	57	308	29	9·4
	A′	A′, —, —, —.	C	7	8·3	3·14		237	53	22·3
IX	LSA	CSA, CS.	T	8	3·3	0·91	79	1,290	182	14·1
	A	A, —.	C	8	6·9	4·25		909	256	28·2
X	S′A′	S′A′, S′.	T	8	5·3	1·83	53	344	65	18·9
	A′	A′, —.	C	7	9·4	3·93		123	53	43·1
XIII	LSA, LSA	—, —.	T	8	5·3	1·33	64	212	57	26·9
	A, A	—, —.	C	8	5·3	3·69		77	37	47·4
XV	*BMA	*BMA, BM.	T	4	4·8	0·00	100	29	1	3·4
	A	A, —.	C	4	5·3	1·75		30	9	30·0
XVI	LSAG	LSAG, LSG.	T	8	2·3	0·59	79	114	11	9·7
	AG	AG, G.	C	8	4·8	2·81		76	30	39·5

* Wrongly made, and caused much

† Maximum 100.

‡ Maximum 5.

I. *(Cox's Orange Pippin.)*

Scab.										
Fruits.										
6 el. ffi-ency† of reat-ent.	7 Total Picked.	8 % Picked.	9 % of Picked Infected.	10 Rel. Efficiency† of Treatment.	11 Scab Equiv.	12 Rel. Efficiency† of Treatment.	13 Total Dropped.	14 % Dropped.	15 % of Dropped Infected.	16 Russet Equiv.
79	225 85	52 44	15·6 83·5	81	2·0 36·9	95	208 109	48 56	2·4 14·7	8·6 12·5
77	179 110	47 32	17·9 90·0	80	3·4 26·1	87	203 236	53 68	3·4 22·0	6·6 8·1
72	124 86	29 35	28·2 81·4	65	4·7 29·0	84	306 162	71 65	3·9 17·3	7·5 9·0
95	248 373	54 42	2·8 76·4	96	0·8 24·3	97	211 515	46 58	0·9 10·1	48·5 7·9
64	208 71	39 30	27·4 80·3	66	4·2 24·5	83	321 169	61 70	1·6 13·0	5·3 10·9
58	136 74	44 31	18·4 59·5	69	3·4 19·6	83	172 163	56 69	2·3 5·5	13·4 6·9
50	440 203	34 22	38·4 86·7	56	7·0 28·0	75	850 706	66 78	1·5 11·3	24·5 23·4
56	129 36	38 29	41·1 86·1	52	8·3 18·8	56	215 87	62 71	5·6 25·3	6·1 4·6
43	78 32	37 42	65·4 93·8	30	20·7 23·5	12	134 45	63 58	4·5 15·6	4·2 3·1
89	18 14	62 47	5·6 57·1	90	0·7 13·4	95	11 16	38 53	0·0 6·3	73·9 14·8
75	22 32	19 42	36·4 65·6	45	4·2 15·6	73	92 44	81 58	3·3 20·5	8·6 5·0

amage. See text.

Root-stock.	Treatment.		Treated or Control.	No. of Trees.	Mildew.			
						Leaves.		
	Pre-blossom.	Post-blossom.			Av. % Mildew per tree.	1 Av. Scab Equiv.‡	2 Rel. Efficiency† of Treatment.	3 Total No. Fruits Produced.
I	LSA	LSA, LS.	T	8	3·6	1·25	71	2,390
	A	A, —.	C	8	5·9	4·38		1,059
II	LSA	S′A′, S′.	T	8	0·8	1·50	64	4,392
	A	A′, —.	C	8	4·3	4·13		1,802
III	CSA	CSA, CS.	T	8	2·0	2·88	38	1,647
	A	A, —.	C	8	1·4	4·63		1,371
IV	*BMA	*BMA, BM.	T	4	1·3	1·00	71	2,149
	A	A, —.	C	4	1·3	3·50		2,097
V	LSA, LSA	S′A′, S′.	T	8	1·7	1·00	75	3,965
	A, A	A′, —.	C	8	6·4	4·00		1,971
VI	S′A′	S′A′, S′, S′, S′.	T	8	0·4	2·00	46	1,157
	A′	A′, —, —, —.	C	7	2·2	3·71		1,026
IX	LSA	CSA, CS.	T	8	1·1	2·25	53	2,642
	A	A, —.	C	8	3·4	4·75		1,853
X	S′A′	S′A′, S′.	T	8	1·2	3·38	19	1,346
	A′	A′, —.	C	7	3·8	4·14		612
XIII	LSA, LSA	—, —.	T	8	0·6	1·00	75	1,870
	A, A	—, —.	C	8	2·3	4·00		938
XV	*BMA	*BMA, BM.	T	4	1·4	0·75	77	470
	A	A, —.	C	4	1·6	3·25		580
XVI	LSAG	LSAG, LSG.	T	8	0·6	1·25	70	1,383
	AG	AG, G.	C	8	1·9	4·13		1,663

* Correctly
† Maximu
‡ Maximu

TABLE II.

pple Mildew, 1932. (*Cox's Orange Pippin.*)

Scab.												
Fruits.												
4 Total Scab-infected.	5 % Scab-infected.	6 Rel. Efficiency† of Treatment.	7 Total Picked.	8 % Picked.	9 % of Picked Infected.	10 Rel. Efficiency† of Treatment.	11 Scab Equiv.	12 Rel. Efficiency† of Treatment.	13 Total Dropped.	14 % Dropped.	15 % of Dropped Infected.	16 Russet Equiv.
540	22·6	67	750	31	33·6	61	6·0	80	1,640	69	17·6	39·7
722	68·2		456	43	87·0		30·6		603	57	53·9	57·5
762	17·3	71	753	17	29·7	67	5·9	80	3,639	83	14·8	22·9
1,059	58·8		380	21	89·7		30·0		1,422	79	50·5	54·2
838	50·9	26	459	28	65·4	28	15·0	59	1,188	72	45·3	58·0
942	68·7		332	24	91·3		37·0		1,039	76	61·5	36·4
1,002	46·6	38	1,170	54	45·2	48	17·4	52	979	46	48·3	16·2
1,579	75·3		784	37	87·1		36·0		1,313	63	68·2	28·6
515	13·0	78	997	25	24·3	74	4·3	87	2,968	75	9·2	24·2
1,175	59·6		500	25	93·8		34·0		1,471	75	48·0	45·0
249	21·5	67	441	38	27·7	64	6·3	71	716	62	17·7	53·2
667	65·0		410	40	76·8		21·9		616	60	57·1	41·7
632	23·9	61	476	18	38·4	57	6·5	79	2,166	82	20·7	31·3
1,149	62·0		294	16	89·5		31·0		1,559	84	56·8	50·9
366	27·2	58	456	34	44·1	50	7·4	74	890	66	18·5	43·1
394	64·4		205	33	88·8		28·4		407	67	52·1	37·3
472	25·2	62	737	39	46·8	46	7·8	73	1,133	61	11·2	23·2
625	66·6		205	22	86·3		28·7		733	78	61·1	48·8
153	32·6	54	213	45	34·7	60	8·3	72	257	55	30·7	6·0
418	70·3		148	35	85·8		30·0		432	65	67·4	18·3
496	35·9	48	164	12	43·9	51	7·9	77	1,219	88	34·8	17·7
1,142	68·7		293	18	89·4		34·7		1,370	82	64·2	37·5

made and caused no damage.

n 100.

5.

merely conjecture, and needs experimental verification. It is clear that methods of commercial dusting would vary considerably according to local conditions.

That dust has the property of drifting out of control for considerable distances gives rise to two important considerations which cannot be too strongly urged. It is well known that certain apple varieties, notably Stirling Castle, Lane's Prince Albert, Newton Wonder, Rival, Belle de Boskoop and St. Cecilia are very liable to injury from sulphur. Dusting with sulphur would be highly undesirable, therefore, in a plantation containing any of these varieties. Furthermore, the inclusion of a poison such as lead arsenate for the control of biting insects might be attended with regrettable results to stock, such as sheep and cattle, in the vicinity, and even to human beings should the drift contaminate food in nearby houses, although the experiments of Rancourt and Dupois (11) suggest that this possibility is remote. Nevertheless, such points as these must be borne in mind when dusting is being considered for Scab-control, apart from the efficacy of the dust as a fungicide.

RESULTS.

All the data obtained in 1931 are classified in Table I. The crop was small, and varied considerably from tree to tree in the same treatment, partly because of a severe attack of Apple Blossom Weevil. In view of this, all fruits from similarly-treated trees in the same experiment were classed together in calculating results.

The following symbols are used in Table I and Table II :

LS —Lime-sulphur.
BM —Bordeaux mixture.
CS —Colloidal sulphur.
S′—Sulphur dust.
A′—Lead arsenate dust.
A —Lead arsenate paste.
G —Spray gelatine.

Columns of figures relating to Apple Scab are numbered from 1 to 16 for convenience in reference.

DISCUSSION OF RESULTS.

General.

It should be stated here that the " half-strength " Bordeaux mixture applied at pink-bud and petal-fall was wrongly made. This was evident from the severe foliage-injury caused by the spray. It was found that, owing to a misunderstanding, the hydrated-lime suspension, instead of the Bordeaux mixture, had been strained through butter-muslin, so that the mixture was deficient in lime. This was rectified for the second post-blossom application, and there was no further injury. Results obtained in 1931 with Bordeaux mixture, however, cannot be regarded as conclusive.

The number of days when spraying and dusting could be well done at the right time were very few. Applications had to be made under conditions far from ideal, and, although they were all made in dry weather, strong winds were often encountered, especially early in the season, at green-bud, pink-bud and petal-fall. Uneven cover, and drenching of one side of the tree, were inevitable, and some marginal leaf-scorch resulted from the green-bud and pink-bud lime-sulphur sprays. This had no serious consequences. Furthermore, spray- and dust-deposits were subject to heavy washing by frequent rains that fell after the blossom period. Very wet weather during late July and in August was very favourable for Scab-infection and its development, and much late infection occurred generally.

The eleven experiments are listed according to the number of the rootstock concerned: thus, on stock No. IX, reference is made to Experiment (Ex.) IX, and so on. The experiments were planned so that comparisons between the results of certain of them reveal the effect of a variation in treatment; for example, Ex. I compared with Ex. II shows the relative merits of post-blossom spraying and dusting, the pre-blossom treatment being the same. The handicap (previously referred to) provided by the variation in rootstock lies in the possibility of there being a differential reaction to treatment between one stock-scion combination and another. This has already been hinted in connection with manurial treatments (3) and with the control of Pear Scab (8, 9), and must be borne in mind in interpreting these results.

Apple Mildew.

The degree of infection was practically negligible, which is, evidently, a tribute to the value of cutting out in Winter and Spring as a measure of control. In previous seasons, when infections have been allowed to remain from year to year (except those normally removed by winter pruning), mildew has accumulated, especially on unsprayed trees, and the beneficial effect of sulphur sprays has been shown, although infection was never really severe. Of control in 1931, however, little can be said except that, in a year of slight infection, sulphur sprays and dusts reduced the incidence of Mildew, and that Bordeaux mixture had no beneficial result. Colloidal sulphur alone was somewhat inferior to the other sulphur preparations, while pre-blossom sprays alone (Ex. XIII) did not reduce infection. Trees on stocks No. XVI, XV, XIII and IV respectively were more lightly infected than those on No. V, II and I. All these results support previous findings.

Scab on Leaves. (Col. 1 and 2.)

No Scab was found on Bordeaux-sprayed trees, but, for reasons already given, this result is not a true indication of the fungicidal value of the spray. Three lime-sulphur applications (Ex. I) gave excellent results which were not sustained where gelatine was included (Ex. XVI). Two pre-blossom sprays showed little more effect than one (compare Ex. V and Ex. II). Colloidal sulphur (Ex. III and IX) was less effective than lime-sulphur (Ex. I) and more expensive, especially where used pre-blossom as well as post-blossom. Sulphur dust, though also less effective than lime-sulphur, proved useful post-blossom following lime-sulphur spraying pre-blossom (Ex. II and V), but failed where used both pre- and post-blossom (Ex. VI and X). Failure to obtain early control on the leaves was not compensated by extra post-blossom dustings in this predominantly wet summer. A noteworthy result was shown by pre-blossom without post-blossom treatment (Ex. XIII) where control was, relatively, very good.

Control trees on Stocks No. XV, IV and XVI were more lightly infected than those on No. I, II, IX and V. No. XVI induced less infection than formerly, otherwise the results conform with earlier ones.

Total Number of Fruits Produced. (Col. 3.)

This figure is the sum of picked and *all* dropped fruits. Applications of fungicides always resulted in increase of total " set "* (regardless of previous seasons' treatment) except where the wrongly-made and injurious Bordeaux mixture was used. Doubtless, the pink-bud application damaged the blossoms and this impaired setting. In the light of earlier results (7), it is clear that pink-bud spraying (or dusting), which controls Scab and caterpillar and does no damage to the tree enhances fruit-" set ".

Relative Efficiency of Treatments based on Percentage of Infected Fruits in Entire Crop, dropped and picked.† (Col. 4, 5 and 6.)

The efficiency of Bordeaux mixture was outstanding when computed by this method. Thus, the most harmful spray was the most effective. It is frequently noticed that best control is obtained where most damage is done, and it seems that toxic properties in sprays often react on fungus and host alike. It emphasizes the importance of determining that strength of spray which is safe to use while giving the maximum fungicidal efficiency (7). This point has been noted before in connection with soda-soap solution (3).

Fruit-infection occurred much later than leaf-infection on these trees in 1931, and thus post-blossom sprays were of paramount importance to the fruit. Control was poor in Ex. XIII, which showed a much better result on the leaves.

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* Dropped and picked.

† These results are of much more importance from the experimental than the commercial standpoint, for they include infection on all dropped fruits.

Dusting pre- and post-blossom (Ex. VI and X) was not very satisfactory as far as results in Col. 6 are concerned (compare with discussion on Col. 10, below), extra dustings failing to make any difference to the relative number of fruits infected (all trees on No. VI stock were only lightly infected compared with those on No. X). Comparison between Ex. II and Ex. V shows that better control was given by one pre-blossom application than by two. The most likely explanation of such a curious phenomenon is that trees on No. II stock were more easily protected than those on No. V ; in other words, indications (already referred to in this paper) are shown of a differential reaction of rootstock to spray-treatment. Similarly, when Ex. III and Ex. IX are compared, three colloidal sulphur applications show a better result than one lime-sulphur followed by two colloidal sulphur sprays, and practically as good as three lime-sulphur sprays (Ex. I). Yet throughout this investigation of five years' duration, colloidal sulphur at 0·4 per cent. has given less satisfactory results against Scab than lime-sulphur at 1-100. It appears likely, therefore, that trees on No. III stock were more easily protected than those on No. IX, although an explanation is not forthcoming.

It will be noted that, although fewer fruits were produced by control than by treated trees, in nearly every case there was a much greater number of them infected.

Picked Fruits. (Col. 7, 8, 9, 10, 11 and 12.)

The value of spraying is shown by the fact that, in the aggregate, more than double the crop was picked from treated than from control trees. Moreover, although treated trees set a much greater number of fruits than controls, a greater *percentage* of these remained to maturity. Exceptions are shown by Ex. III and XIII (in which the percentage of dropped infected fruits is relatively high, indicating early Scab-infection), and by Ex. XVI, where the percentage of dropped fruits on treated trees is unaccountably high.

Column 10 shows the Relative Efficiencies of treatments on the picked crop. These figures are based on the percentage of fruits scabbed, irrespective of the severity of infection on each fruit. The results closely follow those based on the entire fruit " set " with two noteworthy exceptions. (a) Extra post-blossom dustings now show a beneficial effect (compare Ex. VI and X). This would be expected, for the influence of early-dropped fruitlets, which were not affected by the extra dustings, is eliminated. (b) Ex. XVI shows a very poor result, which seems to be related to an exceptionally high percentage of dropped fruits (mainly Scab-free) on treated trees.

Column 12 shows the results obtained when the same fruits were graded according to the area of Scab-infection on them. Earlier results are entirely supported in that three lime-sulphur applications gave excellent results. Here the relative merits of post-blossom treatments are clearly demonstrated in a season when post-blossom protection was of great importance for effective Scab-control on the fruits. Comparison of results in Ex. I, II, III, V, IX and XVI shows that lime-sulphur was more effective than colloidal sulphur or sulphur dust (see Fig. II, III and IV); that it was better without gelatine than with it, and that a green-bud application was not necessary at East Malling in 1931. For reasons already explained, it may be assumed that differences between the results in Ex. II and V, and between those in Ex. III and IX, mean little. Extra post-blossom dustings were very beneficial to the fruits, if not to the leaves (compare Ex. VI and X) in summer weather favourable to Scab. Where no post-blossom treatment was given (Ex. XIII), the effect of the pre-blossom treatments was practically lost by the end of the season, in contrast to the result shown by the leaves.

Comparison between results in Columns 10 and 12 gives an idea of the type of infection. In most cases it was in the form of small spots (because the Efficiency shown in Col. 12 is generally much greater than that corresponding in Col. 10), for the season was such that an extra post-blossom application would have been of great value if applied at mid-July, as much late infection occurred towards the end of that month. This was fostered by extremely wet weather in August. Extra post-blossom dustings not only kept a greater percentage of picked fruits clean, but they still further checked the development in total area of the Scab-spots. Thus with Ex. VI there is a greater difference than with Ex. X between results based on numerical proportion of fruits infected (Col. 10) and the proportion of surface of fruits infected (Col. 12). A high percentage infection together with heavily-infected fruits is shown by Ex. XIII, where no post-blossom treatment was given either to prevent infection or to check its development.

That it is desirable to base results not only on the percentage of fruits infected but also on the severity of that infection is shown by comparing results in Ex. I and Ex. II. They are similar where area of infection is not taken into account (Col. 10), but the superiority of lime-sulphur over sulphur dust is shown where area is the basis of evaluation (Col. 12).

Rootstock-influence on the incidence of Scab, based on graded infection on fruits on control trees, was not in conformity with that in 1930 (7). Starting from most-infected to least-infected fruits, rootstock-influence ranged in the following order in 1931: No. I, III, IX, II, V, IV, XIII, VI, X, XVI, XV. In 1930, the corresponding range was No. XVI, I, X, VI, IX, II, V, III, XIII, IV. (Trees on No. XV had insufficient crop to be included in that year.) It will be seen that, while the relative position of stock No. I was similar in each year, some of the stocks that induced relative resistance in 1931 induced relative susceptibility in 1930 (e.g. No. XVI, X and VI). The light cropping of trees on stocks No. X, XIII, XV and XVI in 1931, together with different seasonal conditions, must be considered as governing factors in such results. Meanwhile, until more data are forthcoming, a considered statement of the relative influence of each rootstock on susceptibility to Scab on Cox fruits cannot confidently be made.

Dropped Fruits. (Col. 13, 14 and 15.)

These figures are complementary to those concerning picked fruits. When dropped fruits and picked fruits are considered together, no evidence can be found that any of the treatments caused fruit-drop. Although in certain instances more fruits dropped from treated than from control trees, this evidently is due to the much greater " set " on treated trees. The *percentage* dropped is greater, in most cases, on the controls. Evidence of indifferent control when the fruits were very small has already been pointed out in regard to Ex. III and Ex. XIII, where the percentage dropped, and the percentage of dropped infected fruits on treated trees are both relatively high. The same is true of Ex. X, which shows a relatively high proportion of dropped fruits scabbed on treated trees.

Russeting of Picked Fruits. (Col. 16.)

As would be expected in the circumstances already described, Bordeaux mixture caused severe fruit-russeting which rendered many of the fruits unmarketable. In only one other instance, that of trees on stock No. IX, was the russeting severe enough to be regarded as detrimental. In this case the russeting must be associated with rootstock-influence and not with spray-damage, for stock No. IX has previously shown the same tendency (7). The tendency for fruits on control trees to show more russeting than those on comparable treated trees was not so consistent in 1931 as in 1930 (4, 7).

Effect of Previous Spray-Treatment.

Detailed examination of the results in Scab-control on leaves and fruits of individual trees showed a definite cumulative effect of previous years' spray-treatment. Control trees, of course, were the most heavily infected, but, as with treated trees, infection was greater on trees formerly controls than on those formerly treated.

The following examples, chosen at random, illustrate this point.

Cox's O.P. on stock No. IX.

Average Scab-equivalent in 1931 of *fruits* of trees :

Treated in old trials (T) Treated in new trials (T′)	4·3	Controls in old trials (C) Controls in new trials (C′)	57·5
Treated in old trials (T) Controls in new trials (C′)	36·0	Controls in old trials (C) Treated in new trials (T′)	10·8

Cox's O.P. on stock No. III.

Average Scab-equivalent in 1931 of *leaves* of trees:

TT′ 0·9; CC′ 3·9; TC′ 2·6; CT′ 1·5.

This phenomenon may be partly responsible for certain unexplained inconsistencies in present results.

EXPERIMENTS IN 1932.

The experiments were repeated in 1932, the same sprays and dusts being used on the same trees as in 1931.

Times of Spray- and Dust-Applications.

1. Green-bud	April 27th.
2. Pink-bud	May 18th.
3. Petal-fall	June 2nd.
3a. Ten days after 3	June 13th (dust only, on trees on No. VI stock).
4. Three weeks after 3	June 23rd.
4a. Ten days after 4	July 4th (dust only, on trees on No. VI stock).

Notes on Weather Conditions in Relation to Spore-Dispersal and Bud-Development.

The first ripe ascospore was found (in dead leaves kept under natural conditions in a wire cage) on April 18th, but this appeared to be an exceptional instance, for further examination on the same day failed to reveal any more ripe spores, although the contents of the asci were beginning to take definite shape. Ejection of ripe ascospores occurred freely towards the end of the third week and throughout the fourth week of April, when Cox trees in these experiments had scarcely reached green-bud stage. Conidia, also, were being freely produced in the young shoots of control trees. By the first week of May, Scab-infection was prevalent on the leaves surrounding the flower-trusses, which were then at somewhat advanced green-bud.

The period from green-bud (April 27th) to petal-fall (June 2nd) was about thirty-four days, that is, almost exactly the same as it was in 1931. The period from green-bud to pink-bud (May 18th) was twenty days, while that from pink-bud to petal-fall was fourteen days. Thus, in 1932 the longer period occurred between green-bud and pink-bud, while in 1931 it was between pink-bud and petal-fall.

This seasonal difference was due to weather conditions which had a great bearing on the results of spraying. Cold, wet, sunless weather had delayed development of the flower-trusses after bud-break, so that green-bud was at least a week later than usual. Such weather conditions, unsuitable for tree-growth but evidently suitable for development of the fungus, lasted until mid-May, when a sudden burst of hot weather caused the trees to come quickly into flower, the period of pink-bud stage being very shortened.

It was obvious even before the pink-bud application was made that a green-bud application was necessary to control early infection in 1932. This is the first occasion in seven consecutive years (during which these experiments have been made) that spraying at green-bud as well as at pink-bud for Scab-control at East Malling has been really justified. Nevertheless, certain factors met with in commercial practice may make two pre-blossom applications desirable, while in some districts, where local climatic and other conditions are, normally, different from those at East Malling (for example, in the West of England (2)), the earlier pre-blossom spray has become established routine.

The result of late Scab-infection appeared in 1932 during August, when small spots occurred on many fruits, especially round the eye. This infection followed a very wet period towards the latter part of July.

RESULTS.

All the data obtained in 1932 are classified in Table II. The crop in 1932 was much heavier than that in 1931, but, to preserve uniformity between the results in both years, all fruits from similarly-treated trees were classed together, as in 1931, in calculating results. The same symbols for the various sprays are used in Table II as in Table I.

DISCUSSION OF RESULTS.

General.

With the experience of 1931 as a guide, dusting was done in the day-time when the trees were dry and a light wind was blowing. Satisfactory " cover " was obtained and most of the drift was arrested by the hessian screens. The little that escaped, especially that at petal-fall, was sufficient to cause severe leaf- and fruit-drop on interplanted Stirling Castle trees, although the deposit of dust on the leaves was scarcely visible to the naked eye.

Reference has already been made to the sudden burst of trees into bloom, and to the brief pink-bud period. Because of this, many of the Cox trees had a fair sprinkling of open flowers (especially on the lower parts of the branches) before the pink-bud application was made. This was especially noteworthy on trees on stocks No. I and V, those on No. IX being relatively later than usual. Pink-bud sprays and dusts were necessarily applied on a very hot, sunny day, and considerable damage to the lower flower-trusses and their surrounding leaves resulted on trees sprayed with lime-sulphur with arsenate. This may have been connected to some extent with the fact that an application of nicotine and soft soap had been made the day previous against Apple Capsid, resulting in considerable browning of open and unopen petals. Furthermore, a long period of cold wet weather probably affected " leaf-condition ", so that the foliage was less resistant than usual to spray-damage.

Bordeaux mixture (4-18-100) caused no appreciable damage either pre- or post-blossom. It is thus possible to reduce the strength of Bordeaux so that it causes no damage even on Cox's Orange Pippin, but it will be seen that the control of Scab was indifferent.

Colloidal sulphur and sulphur dust caused very little damage when used at pink-bud. They also had little effect on existing Scab-infections, which were very numerous. Lime-sulphur (1-30), however, with or without gelatine, scorched scabbed leaves which subsequently dropped, together with healthy leaves damaged by this spray. Where a green-bud application of lime-sulphur was made, there was less leaf-drop following the pink-bud application than where the earlier spray was not given. In this connection, a striking demonstration of the influence of rootstock on spray-damage was observed. Leaf-drop on trees on stock No. XIII was negligible, while on trees on No. V it was about half as severe as that on trees on No. I, II, IX, and XVI (which received one pre-blossom application only). This observation supports a previous indication of stock-influence on spray-damage (3) when trees on stock No. V showed more leaf-burn than those on No. XIII. Early infection on the leaves was almost entirely prevented by lime-sulphur applied at green-bud, and this partly explains the less-severe damage to leaves by a subsequent pink-bud application. It cannot be the whole explanation, however, for not all dropped leaves were infected. A contributing factor would appear to be the effect of the green-bud application in mitigating damage caused by a pink-bud application. Possible mitigation of " sulphur-shock " has been noted before (3), but it is likely that the effect depends upon some factor at present obscure.

Sprays and dusts were applied in much better conditions than in 1931. Wind and rain did not interfere with the work, although hot sunny weather when the pink-bud, petal-fall and " three-weeks " applications were made was unavoidable. Very rainy weather in May, during which Scab developed extremely rapidly, was followed by very dry weather in June. This tended to favour the action of sprays and dusts by checking development of the disease.

Apple Mildew.

Reference to Table II shows that there was even less infection in 1932 than in the previous year. Control trees were infected to a very small degree, yet in spite of this, trees sprayed with lime-sulphur and those dusted with sulphur showed less infection than controls. Sulphur dust appeared to be even more effective than lime-sulphur, while each controlled Red Spider also. Colloidal sulphur* throughout, however, was not satisfactory, and Bordeaux mixture again gave no control. Trees on stocks No. V, I and II had rather more mildew than those on other stocks, as in 1931.

Scab on Leaves. (Col. 1 and 2.)

The most noteworthy point is that, in all experiments except one, Scab-control was greatly inferior to that obtained in 1931, a result due solely to different seasonal conditions, already discussed. An important exception was shown by Ex. XIII, where in spite of no post-blossom treatment, control was as effective with two pre-blossom sprays alone as with any other treatment. Support is thus given to a former assertion that excellent Scab-control cannot be attained without the *initial* ascendancy of fungicide over fungus (7). Comparison between Ex. II and Ex. V also shows that better control was given where two pre-blossom sprays were applied, especially when results obtained in 1931, suggesting that trees on stock No. II were more easily protected than those on No. V, are borne in mind. Bordeaux mixture gave good control (Ex. IV and XV) and caused no appreciable damage, but reduction in strength of copper sulphate to half impaired the fungicidal value of the spray in comparison with that of the " full-strength ", though harmful, mixture used in the earlier years (3, 7). Three lime-sulphur applications also gave good control (Ex. I) ; equally good results were obtained where gelatine was included (Ex. XVI), although the degree of control was not as high as that attained in 1931. Colloidal sulphur and sulphur dust were each inferior to lime-sulphur whether they were used post-blossom following lime-sulphur pre-blossom (Ex. I, II and IX) or used throughout (Ex. I, III and X), pre- and post-blossom. Indeed, the pre-blossom lime-sulphur spray was instrumental in raising the degree of control from very poor (Ex. III and X) to moderate (Ex. IX and II). Thus, there was greater evidence of its value in 1932, when pre-blossom sprays were so important, than in 1931 when infection occurred relatively later and greater emphasis was laid on the value of post-blossom treatment. Extra post-blossom dustings (Ex. VI) partially compensated for lack of early control, although the final efficiency remained poor. This result, contrary to that obtained in 1931, is doubtless due to the check to Scab-development given by dry weather in Summer 1932, especially in June.

Control trees on stocks No. XV, IV and VI were more lightly infected than those on No. IX, III and I. Trees on No. XV and IV have consistently borne lightly-infected leaves throughout these experiments, while those on No. I, IX, V and II have almost consistently borne more heavily-infected leaves. Trees on the other stocks have normally occupied an intermediate position which, however, changes somewhat from season to season.

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* As pointed out in a paper on the control of Pear Scab (9), this spray might be more effective if used with a good wetter and spreader.

Total Number of Fruits Produced. (Col. 3.)

As in 1931, this figure is the sum of picked and *all* dropped fruits. The crop was very much greater than in 1931, applications of fungicides again resulting, in general, in heavier crops (see Fig. V and VI). The effect on the 1932 crop of severe Bordeaux-damage in 1931 can be seen in Ex. IV and XV. In the former, there is little difference in the numbers of fruit produced by control and by treated trees, while, in the latter, the controls bore many more fruits. No appreciable damage was done by this spray in 1932 for reasons already explained. That control trees on stock No. XVI produced a greater number of fruits than comparable treated trees cannot be explained.

Relative Efficiency of Treatments based on Percentage of Infected Fruits in Entire Crop, dropped and picked. (Col. 4, 5 and 6.)

Bordeaux mixture, formula 4-18-100, gave a disappointing result, especially on trees on stock No. IV, and evidently is too weak in copper sulphate for the spray to be used in a *basic* Scab-control programme. It might be of value if used in the same way as sulphur dust, that is, as an adjunct to the basic programme in certain special circumstances, for, being " safe " even on Cox's, it would not be likely to harm trees of many varieties as the " full-strength " spray does. It is interesting to note that reduction in strength of Bordeaux leads to reduction in fungicidal efficacy, for the same is true of lime-sulphur. Yet it is often asserted that lime-sulphur is fungicidally inferior to Bordeaux (regardless of strengths) although the latter is not so frequently used, partly because it causes more damage ! It may be that, for post-blossom spraying, reduction in strength of Bordeaux to approximately a third of the strength of that used pre-blossom, as with lime-sulphur, would yield results much more fairly comparable with those given by lime-sulphur.

The value of two pre-blossom sprays in this particular season is seen by comparing the results of Ex. V and XIII with those of the other experiments. Two pre-blossom without post-blossom sprays (Ex. XIII) reached, relatively, a very high degree of efficiency, although, possibly, Cox's on No. XIII were more easily protected than those on No. V. Three applications of lime-sulphur (Ex. I) were excelled (see Ex. V) for the first time in this series of experiments (except when Bordeaux was superior but caused spray-injury), though they gave good control. Where gelatine was used as well (Ex. XVI) the result obtained was indifferent, due largely to infection on dropped fruits (Col. 15).

Colloidal sulphur failed where used throughout (Ex. III), but sulphur dust proved very beneficial (Ex. II, VI and X), more so, relatively, than in 1931 when the Summer was fairly wet. Extra dustings caused a slight decrease in the percentage of all fruits infected (Ex. VI and X), though infection occurred before the extra treatment could be very effective.

As in 1931, although treated trees in general produced more fruits than controls, many more of the latter than of the former were scabbed.

Picked Fruits. (Col. 7, 8, 9, 10, 11 and 12.)

The evidence provided by the figures showing percentage of fruits picked (Col. 8), coupled with general observation in the plantation during the season, shows that post-blossom applications of lime-sulphur (Ex. I) and of sulphur dust (Ex. II, V, VI and X) caused fruit-drop. Sulphur dust has previously been known to cause damage, leaf-drop occurring on Belle de Boskoop in 1929 (3). There is no clear evidence that colloidal sulphur (Ex. III and IX) or the " half-strength " Bordeaux mixture (Ex. IV and XV) caused similar injury. The result of Ex. XVI must be regarded with doubt, for there was a high percentage fruit-drop on these trees also in 1931.*

The above inference can be based on the result of Ex. XIII, where no post-blossom treatment was given and where the percentage of fruits picked from treated trees was much greater than that from the controls. If no fruit-drop were caused, this would be expected, as shown by many former data (7). The results of post-blossom Bordeaux spraying (Ex. IV and XV) and, to a less degree, of post-blossom colloidal sulphur spraying (Ex. III and IX) conform to those of Ex. XIII. Where lime-sulphur or sulphur dust was used post-blossom, however, the percentage of fruit picked on treated trees fell to the level of that on controls and, in certain cases, even below. There was no evidence of fruit-drop in 1931. The Summer of 1932 was more conducive to damage by sulphur preparations than that of 1931, and it will be recalled that spraying and dusting were necessarily done in hot, sunny weather. Nevertheless, the fact that the trees were bearing much heavier crops in 1932 than in 1931 must not be forgotten, for it is possible that the drain on a tree as a result of setting a heavy crop may affect its resistance to spray-damage.

Results in Scab-control, when based on percentage infected of picked fruits (Col. 10) were again generally similar to those based on percentage infected of all fruits (Col. 6). Ex. XIII, however, shows an inferior result which is not unexpected when efficient early control is recalled. Small, dropped fruitlets, relatively free from Scab (see Col. 15), were not considered in obtaining this figure, while taking them into account in the results shown in Col. 6 raised the efficiency figure there.

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* It has been observed that Cox's on stock No. XVI have produced their fruits, up to the present in these experiments, mainly from axillary flower-trusses. Such fruits drop very readily. This is the most likely explanation of the puzzling results obtained from trees on this very vigorous stock. Up to 1932, they had not reached full bearing, having grown mainly to " wood ", but when the crop is borne chiefly on naturally-formed spurs as the trees grow older, they will conform more nearly to those on other rootstocks.

With results based on the area of Scab-infection (Col. 12), control in 1932 was not as good as that in 1931, with few exceptions. Clearly this is evidence of the need for regulating the spray-programme according to seasonal conditions. It also shows how important it is to prevent those early infections which have a cumulative effect *on the tree* as the season advances. The 1931 Summer was much more favourable for the development of Scab-infection than that of 1932. Yet, because the disease was prevalent prior to pink-bud in 1932, ultimate results from the same programme of treatments were prejudiced. A study of two exceptions " proves the rule " in this case. In spite of no post-blossom treatment in Ex. XIII, the control was good in a season when early pre-blossom treatment was essential to the maintenance of excellent control by measures adopted post-blossom. The corresponding result was poor in 1931, a season when post-blossom treatment was relatively more important. Two pre-blossom sprays were given in Ex. V also, and the result was outstanding. Extra post-blossom dustings had no beneficial effect in causing reduction of the fruit-area* scabbed (Ex. VI and X). In 1931, however, that effect was very marked. Relatively good control was given in all experiments where lime-sulphur was used only once pre-blossom (Ex. I, II, IX and XVI) whether the post-blossom treatment was lime-sulphur, colloidal sulphur or sulphur dust. Gelatine made no appreciable difference to the effectiveness of lime-sulphur as far as fruit-area infected was concerned (Ex. XVI). A contrary result was shown in 1931. The key to the explanation of disparity in many of these results is to be found in the results in Col. 15, which shows the percentage of dropped fruits infected. Dropped fruits were much more heavily scabbed in 1932 than those in 1931, because infection occurred very early in 1932. Hence, although any one treatment may show good results when these are based on picked fruits only, it does not necessarily follow that good results will be reflected in those data which include the dropped fruits (Col. 6). As the weather conditions in the two seasons so largely governed the time of infection, the balance of results shown in the different columns was largely affected also. A big difference between one season and the other in results based on one method of evaluation can usually be interpreted by the reaction shown in the data of another, corresponding, column. This is an argument for the expression of the results of spraying trials in as many different ways as the data collected will allow. It also points to the need for all the data that can reasonably be obtained.

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* It must be borne in mind in comparing what appear to be anomalous results on fruits and leaves, that new leaves are constantly being produced and these can be kept clean in a moderately dry season by extra dustings. The one crop of fruit, however, if scabbed early, must remain so.

Neither colloidal sulphur used throughout (Ex. III) nor Bordeaux mixture on trees on stock No. IV was satisfactory. Trees on stock No. XV, however, showed a better result. This stock has formerly induced relative resistance to Scab in Cox's Orange Pippin, and trees worked on it might therefore be easily protected.

Comparison between results shown in Col. 10 and those in Col. 12 points to the extent of infection. Low efficiency in Col. 10 coupled with relatively high efficiency in Col. 12 indicates that a high proportion of fruits were lightly infected, and vice versa. A noteworthy instance is shown by trees on stock No. XIII. Late infection attacked many fruits, but the total area of infection was relatively small. As no post-blossom treatment was given, the lasting effect of early Scab-control in a moderately dry Summer is evident. Comparison between results in Ex. VI and Ex. X shows that extra dustings caused reduction in the percentage of picked fruits scabbed but not in the relative area scabbed. This curious result might be achieved if extra dusting, by checking severe *early* infection, preserved *on the tree* those early-scabbed fruits that normally drop on account of severity of infection, while giving better control of later infection (in the form of small spots) than that given by fewer dustings.

Reference has been made in a paper on Pear Scab (9) to the relationship noted between percentage and area of picked fruit infected on Bordeaux-sprayed trees and on those sprayed with sulphur preparations. Reasons are tentatively offered for the greater prevalence of heavily-infected fruits on Bordeaux-sprayed trees. It will be noted (Ex. IV and Ex. XV) that the same tendency is shown in the present figures, concerning apples, in 1931 and in 1932. Efficiency shown in Col. 10 is not coupled with relatively higher efficiency in Col. 12 with Bordeaux mixture as with sulphur preparations (with few, readily-explained exceptions).

The influence of rootstock on (graded) Scab-infection on control trees (Col. 11) in 1932 is shown by the following range, from most-infected to least-infected fruits: No. III, IV, XVI, V, IX, I, II, XV, XIII, X, VI. No. I induced relatively less susceptibility than formerly, while No. IV induced relatively more than formerly.

Dropped Fruits. (Col. 13, 14 and 15.)

These figures must be considered in conjunction with data concerning the crop picked before their full significance can be appraised. They support the contention to be found earlier in this paper that post-blossom applications of lime-sulphur and of sulphur dust caused fruit-drop. Ex. XIII, for this purpose, acts as a " control ". It has already been noted that dropped fruits, both on treated and on control trees, were much more heavily infected in 1932 (owing to severity of early infection) than in 1931. Where two pre-blossom sprays were given (Ex. V and XIII), dropped fruits were only lightly infected.

Russeting of Picked Fruits. (Col. 16.)

Severe russeting was general throughout the plot in 1932, and it is attributed to spraying with nicotine and soft soap for capsid-control within a short period of the application of the experimental sprays and dusts, which all contained lead arsenate. This contact spray was applied to all trees pre-blossom, on May 17th, in warm, sunny weather and the pink-bud experimental sprays were given the day afterwards (10). Severe russeting also occurred on many varieties on the farm (Lane's, for example—see Fig. VII) where the lime-sulphur and lead arsenate spray was used pre-blossom against Scab and caterpillar as a routine measure, and was followed in some cases more than, and, in some, less than a week afterwards, still pre-blossom (on May 17th), with nicotine and soft soap for capsid. It is considered that the fruit-russeting in each case was caused by interaction on the tree of these two pre-blossom* sprays. Reaction between lead arsenate (or its derivatives) and soft soap probably resulted in the liberation of " free " arsenic which " burnt " the skin of the young fruits.† It should be noted in the figures that, wherever lime-sulphur or Bordeaux mixture was used pre-blossom (Ex. I, II, IV, V, IX, XIII, XV and XVI), there was less russeting on treated trees than on controls. Where colloidal sulphur or sulphur dust was used pre-blossom (Ex. III, VI and X), there was more russeting on treated trees than on controls. This suggests that sprays containing lime, a strong base, neutralized to some extent the harmful effects of the reaction between the soft soap and lead arsenate. These data indicate the necessity for finding a suitable substitute for soft soap as a wetter and spreader on trees where it is likely to react with lead arsenate. Work is already in progress on a field scale to test sulphite lye as a wetter and spreader and to determine whether, by its use, a contact insecticide can effectively be incorporated with a fungicidal spray, thus obviating the necessity for separate applications, and so economically reducing the cost of spraying.

SUMMARY AND CONCLUSIONS.

Field trials of sprays and dusts against Apple Scab and Apple Mildew on Cox's Orange Pippin were carried out in 1931 and 1932. These are described and the results are fully tabulated and discussed in relation to weather conditions. Certain opinions are offered concerning methods of expressing and interpreting the tabulated results. Observations on experimental dusting methods are also noted.

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* Fruit-russeting has formerly resulted from the *pre-blossom* use of Bordeaux mixture on Cox's Orange Pippin (3).

† This view is supported by Dr. W. Goodwin, of Wye College, Kent, whom the writer asked for advice which is here gratefully acknowledged.

Former results are largely supported, though, for the first time during the last seven years, seasonal conditions rendered two pre-blossom sprays necessary for excellent Scab-control at East Malling in 1932. A " half-strength " Bordeaux mixture caused no damage in 1932, but it gave indifferent control of Scab. Colloidal sulphur, less effective than lime-sulphur, gave good control in 1931 but not in 1932. The results obtained with sulphur dust appeared to depend largely on seasonal conditions. It was useful post-blossom as an adjunct to wet spraying pre-blossom, but was unreliable when used both pre- and post-blossom. It was very effective against Apple Mildew and also controlled Red Spider. Extra post-blossom dustings, *as protective agents*, were beneficial in the generally wet Summer of 1931, and normally it would be advisable, where dusting is practised, to make more applications than would be made where spraying is relied upon. In any case, the assembled data, when read in the light of prevailing seasonal conditions and the time of Scab-infection, all point to the inference that, for dusting to be most effective, it is highly desirable to make applications in advance of infection. Lime-sulphur was the best fungicide (at the strengths used) of those tested ; it gave good control of Red Spider and of Apple Mildew as well as of Apple Scab. With gelatine it was, on the whole, less satisfactory against Scab.

Fruit-drop resulted in 1932 but not in 1931 from post-blossom use of lime-sulphur and of sulphur dust.

The influence of rootstock on Scab-infection on leaves and fruits was noted. Some evidence was obtained that Cox trees worked on certain stocks might be more easily protected by spraying or dusting than those on other stocks.

Severe fruit-russeting was caused in 1932. This is regarded as the result of interaction on the trees, pre-blossom, of soft soap with lead arsenate (or its derivatives).

Certain results are used to illustrate the cumulative effect of treatment in former trials on the same trees.

ACKNOWLEDGMENTS.

The writer is greatly indebted to Miss J. B. Hamond for considerable assistance given from time to time in the field work ; also to Dr. H. Wormald for helpful criticism of the manuscript.

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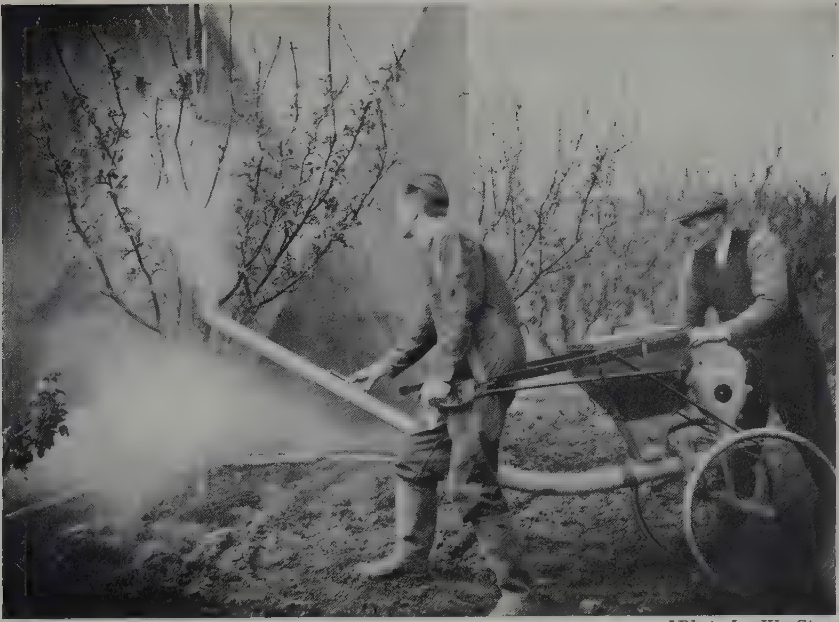

[*Photo by W. Steer*

FIG. I.

Dusting with sulphur at pink-bud, May 6th, 1931. Note the dust drifting and the large hessian screens to arrest the drift.

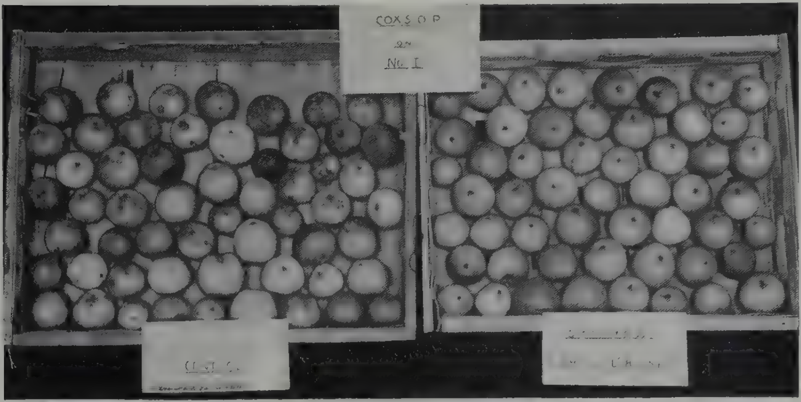

FIG. II.

Random samples of apples showing the degree of control given in 1931 by three lime-sulphur sprays, one pre- and two post-blossom. The relative efficiency (max. 100) represented here is 95. (See Table I, Ex. I.)

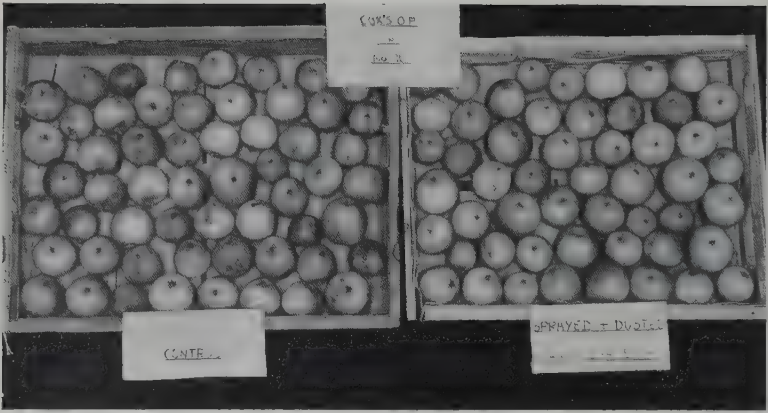

FIG. III.

Random samples of apples showing the degree of control given in 1931 by one pre-blossom lime-sulphur spray and two post-blossom sulphur dusts. The relative efficiency (max. 100) represented here is 87. (See Table I, Ex. II.)

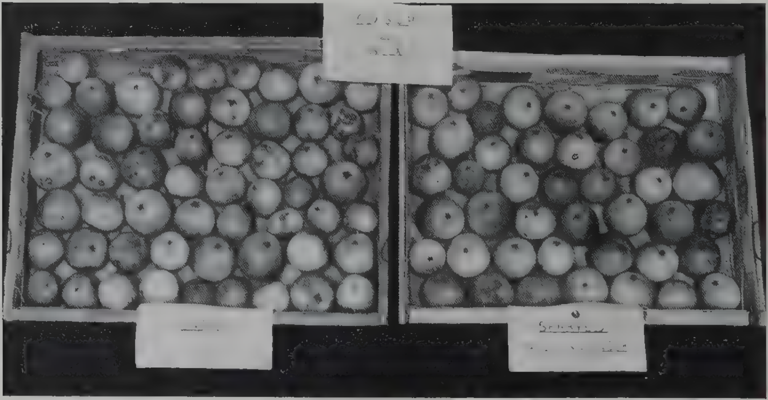

FIG. IV.

Random samples of apples showing the degree of control given in 1931 by one pre-blossom lime-sulphur spray and two post-blossom colloidal sulphur sprays. The relative efficiency (max. 100) represented here is 75. (See Table I, Ex. IX.)

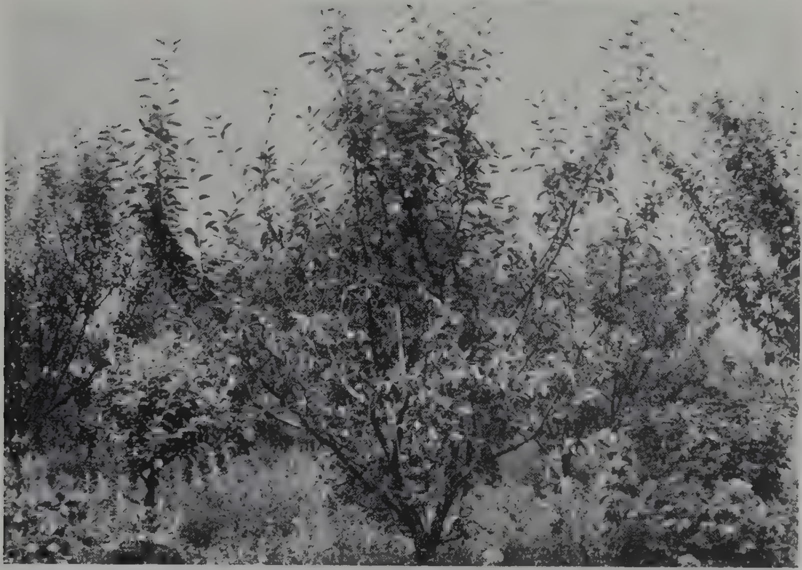

Fig. V.

A " control " Cox tree on No. I stock in August 1932. Note " thin " foliage and virtual lack of crop. Sprayed with lead arsenate suspension in water at pink-bud and at petal-fall. No control of Scab or Red Spider.

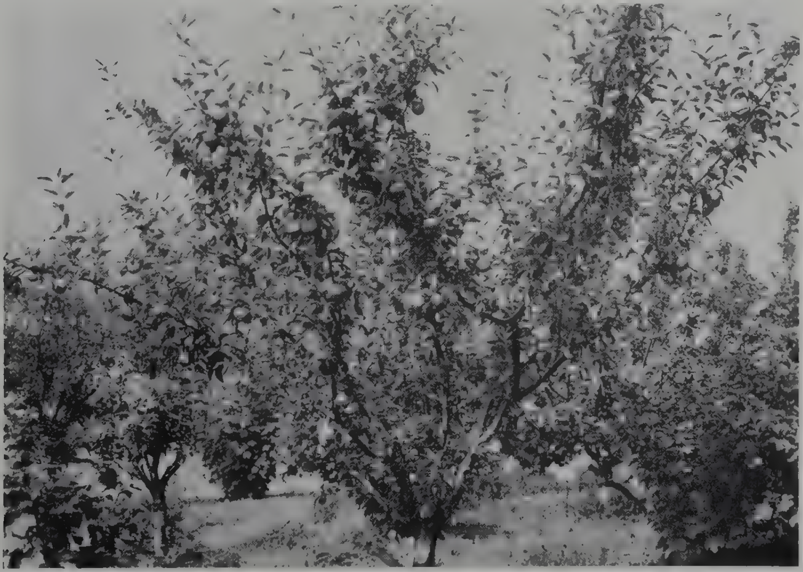

Fig. VI.

A sprayed Cox tree on No. I stock in August 1932. Note luxuriant foliage and good crop, even after some fruit-drop had occurred (see text). Sprayed once pre- and twice post-blossom with lime-sulphur (with lead arsenate paste in first two). Good control of Scab and Red Spider. The relative efficiency (max. 100) represented here is 80. (See Table II, Ex. I.)

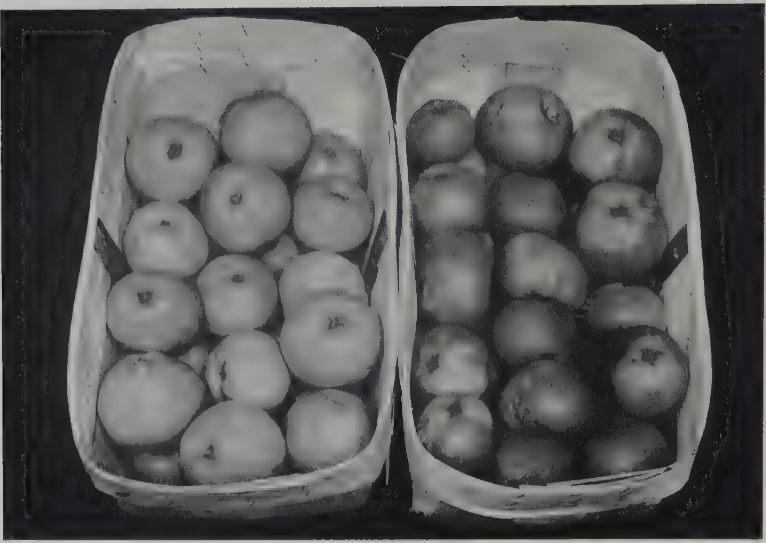

Fig. VII.

Random samples of Lane's Prince Albert apples, 1932. Those in the left-hand chip were sprayed once, pre-blossom, with lime-sulphur and lead arsenate, while those in the right-hand chip received this spray followed, pre-blossom, by nicotine and soft soap. Neither sample was sprayed post-blossom. Note excessive russeting (see text).

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(*Received* 9/12/33.)

BOOK REVIEW

THE HORTICULTURAL EDUCATION ASSOCIATION YEAR BOOK, Vol. II, 1933. 3s. 6d.

The second volume of the Year Book of the Horticultural Education Association fulfils in every way the promise of the first, and has, perhaps, even more meat on its bones. The Editor must be congratulated upon casting his net so skilfully.

Mr. N. B. Bagenal leads off with his Presidential Address, his subject being that great horticulturist Thomas Andrew Knight, a class of man which our English soil has a happy knack of producing.

Commercial Horticulture in Lincolnshire is ably dealt with by Messrs. Murray, Wakerley & Wallace, and provides a good answer to those townsmen who consider the modern farmer still sunk in the apathy of his forefathers. Lincolnshire, now largely the vegetable garden of England, provides a remarkable illustration of the adaptability and enterprise of our cultivators to-day, and promises to be in the near future the bulb garden of England. What would Cobbett, with his dislike of potatoes, say were he to visit Lincolnshire to-day and stalk through its fourteen thousand acres of his detested tubers !

Passing to Scotland, another potato ridden country, we come under the skilful guidance of Mr. D. V. Howells through the fertile Clyde Valley where Victoria plums excel, to the famous raspberry fields of Blairgowrie and the strawberries of the Lothians. We miss any account of the cultivation of runner beans, an important crop in Southern England, but I believe the Scots grow these only for exhibition, not for consumption, a curious commentary upon the time it takes for a new vegetable to become popular.

In persuading Mr. F. A. Secrett to reveal the modes and methods of his great success as a grower of early vegetables the Association has scored greatly, and it is gratifying that horticulturists are so willing to disclose their discoveries to all. Trade secrets in gardening are more often than not pure mythology, thanks to such generous disclosures. This article will be read and re-read by all, even the small garden man will learn much from it, for example, that lettuce with a high nitrogen supply will not suffer from drought, and ground hoof for a top dressing is preferred to nitrogen salts.

Dr. Salaman tells us of the present position of Virus disease in Potatoes and rightly laments the small amount of money available for research. Here is a disease which might quite possibly, as in Conington's thriller, make many parts of the world uninhabitable, and on which we spend a few thousands only in the search for preventive means.

The President starts a brisk heretical breeze blowing through our Cherry Orchards by calling in question the classical sheep and grass methods of cultivation. The heresy, however, of one country is often the orthodoxy of another, and if, on his hoped for voyage in other countries to find better cherries, the writer visits Provence he will there find the cherry grown in the wheat fields, at Pistoia, in Italy, with maize as a cover crop, and at Werder, near Potsdam, on a light sand which would not grow anything but asparagus.

His appeal for an explorer to comb Europe for better varieties is timely. There must be many in Eastern Europe which have never come under the eye of a systematic Pomologist. The writer, however, has searched Central France, Northern Italy and Germany and has at least found a larger and earlier " Early Rivers " among a few dozen hopeful emigrants.

To the rest of the authors in this publication apology is due. The fact that all books have a beginning, a middle and an end leads a reviewer to expand himself unduly on those subjects which come first and which are nearest to his own pre-occupations. We can, therefore, but name the excellent and important articles which follow. Nitrogen and Apples by W. G. Kent touches very closely all those who have put their money on Cox's Orange, and Drs. Kidd and West draw still closer the scattered threads of the Apples' behaviour under storage. Mr. A. J. Woolridge topically treats fruits for canning and Mr. Pearl, no less so, brings the Movies into our quiet horticultural world. Weed Killers of the chemical order are discussed by P. M. Woodman. Some eight pages are devoted to book reviews, an excellent index of the questing spirit of our age, an age when Bacon's wise words, that " Science is not so much an opinion to be held as a work to be done ", have been happily accepted and still more happily acted upon.

E.A.B.

VARIATION IN THE FRUITS OF WASHINGTON NAVEL ORANGES WITH REFERENCE TO THE STANDARDIZATION OF QUALITY BY MEANS OF THE SUGAR/ACID RATIO

By P. R. v. d. R. COPEMAN, B.A., D.Sc.
Government Laboratory, Johannesburg

INTRODUCTION.

THE great development of the fruit industry within recent years has led to a corresponding interest in the composition of fruits and in the changes which occur during growth and storage. In this connection the palatability of the fruits forms an important aspect of the problem and, in studying this question, attention is usually focussed upon the sugar and acid content of the juice, since the characteristics of taste seem to depend principally upon these two constituents. Sweetness may be regarded as a desirable quality in a fruit and it is well known that the intensity of the sweetness may be considerably modified by the presence of an acid or sour substance. It may therefore be concluded that the palatability of the juice, and, consequently, the quality of the fruit, must be closely related to the relative proportions in which the sugar and acid occur in the juice. Since these two factors usually exhibit definitely marked changes in concentration during the development of the fruit, the ratio between them must be closely related to the degree of maturity of the fruit. The importance attached to this ratio in the case of fruits is clearly evident from its extended employment as a measure of quality and ripeness. The practical importance of the sugar/acid ratio can be ascribed to the fact that it embodies an effort to determine the quality of the fruit by characters which are capable of quantitative measurement. When decisions are based entirely upon taste alone, errors of judgment may arise chiefly from the fact that a higher or a lower sugar content is frequently confused with a decreased or an increased acid content. It is, therefore, justifiable to attempt to eliminate such errors by employing quantitative measurements for the more important factors such as sugar and acid. At the same time it is frequently overlooked that the value of the ratio is primarily based upon the assumption that at any given stage of maturity, a definite equilibrium is set up between the different constituents in the juice of the fruit.

In South Africa the sugar/acid ratio has become specially significant in connection with the export of oranges. Oranges are characterized by the fact that they may be gathered for market over a period of two months or more, especially if appearance is the only factor to be considered. During this period the fruit becomes sweeter on account mainly of the decrease in concentration of the acid. The sugar/acid ratio forms an index of these changes and has, therefore, been adopted as a criterion of the degree of ripeness of the fruit. In order to prevent the shipping of green, immature fruits, regulations have been promulgated at different times in which the minimum permissible limits for the different varieties of oranges have been laid down. In commercial practice the soluble solids are generally taken as a measure of the sugar content on account of the greater ease and rapidity with which the former can be determined by using a hydrometer. In this case, however, one term of the ratio consists of all the soluble compounds, including the acid, while the second consists only of the acid and, therefore, the magnitude of the ratio is increased. If due account be taken, however, of this change, the resulting formation will have the same value as a measure of the equilibrium between the constituents of the juice under the conditions of the experiment.

In a fruit, such as the orange, the individual variations are large and there seems to be little information concerning the effect of such variations upon the sugar/acid ratio. A knowledge of the range of variations to be expected in the value of the ratio on account of random sampling as well as of the changes in the ratio due to the degree of maturity are essential in order to judge the significance of the results. In addition it is essential to have some clear idea of the inter-relationships between the soluble solids, the sugar and the acid content of the juice of oranges, since the existing methods of assessing the quality of the fruit depend upon such relationships.

MATERIAL.

The results of an investigation of the changes occurring in the composition of oranges during ripening have been reported in another paper (2). During the final period of ripening the sugar content of the juice increased while the acid content decreased. At full maturity both these factors tended to reach limiting values, after which the changes became small and practically negligible. In this work Washington Navel oranges were employed, being obtained from the Bathurst District of the Eastern Province. The fruit was picked from six different trees which were selected as representative of the orchard. A series of analyses of twenty-five to thirty oranges was carried out at approximately weekly intervals over a period of about three months. During the course of the work a large number of analyses of individual oranges was obtained, and these observations provide data for a critical consideration of the various factors influencing the probable error of the observations made in commercial practice. The data, in addition, throw some light upon the influence of internal and external factors upon the quality of the fruit. Finally, they provide information upon which can be based suggestions for modifying or simplifying the procedure in commercial practice.

METHODS.

The analyses included, amongst others, the determinations of the soluble solids, the sugars and the acid in the juice.

(a) *Determination of Sugar and Acid.*

The method of estimation of the sugar in the juice has been fully described elsewhere (2) and is essentially that of Lane and Enyon (9). For the purposes of the present paper the total sugars were expressed in terms of grams of invert sugar per 100 c.c. juice. For practical purposes this is a more convenient mode of expression and, when comparing one set of samples with another, the error involved may be neglected. The probable error of a single determination was found to be ± 0·3 per cent. of the reading.

The acidity was determined in the usual way by diluting a known volume of the juice and titrating with N/10 caustic soda, using phenolphthalein as indicator. The results were expressed in terms of grams of citric acid per 100 c.c. juice. The greater part of the acids in oranges consists of citric acid and the error involved in expressing the results in this form is negligible. The probable error of a single determination was found to be ± 0·4 per cent. of the reading.

The experimental errors are, therefore, of the same order of relative magnitude in the case of these two factors, and their ratio can obviously be determined with a high degree of accuracy in any particular case.

(b) *Determination of the Soluble Solids.*

During the present investigation the soluble solids were obtained from the density of the juice which was determined by means of a 50 c.c. specific gravity bottle and corrected to 20° C. by means of Tables. The concentration in terms of grams per 100 c.c. was calculated from the expression

$$C = \frac{1{,}000(D - 1{\cdot}000)}{3{\cdot}85}$$

This formula has been shown to give results which are in agreement with the figures experimentally determined in the case of other fruits (5, 1). They also agree with the figures obtained from Tables for the soluble solids per *100 grams* of juice. When estimated in the way the probable error of a single determination has been shown to be ± 0·11 per cent. An additional source of experimental error may be introduced by failure to clarify the juice completely, but this does not amount to more than 1·0 per cent. of the actual reading. The temperature-correction is, however, necessary for an accurate estimation of the soluble solids and the absence of such a correction may give rise to errors of as much as ± 5·0 per cent. depending on the amount by which the temperature of the juice differs from the standard temperature. It is clear, however, that all the factors can be experimentally determined with a high degree of accuracy under suitable conditions.

SAMPLING ERRORS.

Very little information seems to be available concerning the variations in the composition of oranges from random sampling. In order to ascertain the extent of these variations and to obtain some idea of the influence of maturity, the standard deviations were calculated for the soluble solids and the acidity for each successive weekly set of samples. The range of variation compared with the mean value can be obtained from the co-efficient of variation. Owing to the very close relationship between the sugars and soluble solids it may be assumed that the former factor will exhibit the same relative range of variation as the latter. In addition the soluble solids are of more importance from the point of view of commercial practice and it has, therefore, been felt unnecessary to determine the standard deviation for the sugar content. The results obtained are given in Table I.

TABLE I.

		Soluble Solids.		Acid.	
Time in Days.	Number in Sample.	Standard Deviation.	Co-eff. of Variation.	Standard Deviation.	Co-eff. of Variation.
0	28	1·823	14·3	0·351	19·8
12	27	1·166	9·6	0·273	14·9
19	22	1·010	8·1	0·401	23·2
26	22	1·481	11·5	0·298	20·1
33	28	1·182	9·3	0·260	16·2
41	26	1·363	10·3	0·299	18·3
48	27	1·487	10·9	0·253	16·1
62	19	1·080	8·2	0·237	15·7
69	27	1·107	8·3	0·232	15·3
76	24	1·535	11·3	0·279	19·6
83	10	1·015	7·3	0·176	12·8
89	26	1·616	11·5	0·279	20·0
97	26	1·222	8·5	0·223	16·0
104	21	0·771	5·1	0·165	11·6
	Average ..	1·276	9·6	0·266	17·1
	"t"	1·32	2·10	4·06	1·73

The changes in the variations during the final ripening period may be tested by the "t" method of Fisher (8). For a probability of twenty to one against the results of chance effects the value of "t" should be 2·18. It will be seen that the absolute and relative range of variation in the case of the soluble solids shows no significant change and the average values may be taken to represent the normal variation in any random sample of oranges gathered in the usual way for marketing. In the case of the acidity the standard deviation shows a distinct tendency to decrease with time while the co-efficient of variation remains practically constant. From the amounts of the changes in these two factors such a result might be expected. The change in absolute magnitude of the acidity is relatively greater than in that of the soluble solids, so that, as maturity is reached, the tendency to approach the final limiting value in the case of the acidity is more marked. At the same time the average co-efficient of variation may be taken as representing the normal value for any random sample of oranges. The co-efficient of variation in the case of the acidity is seen to be very nearly double that of the soluble solids, so that the average acidity of any sample of oranges is affected to a greater extent by sampling errors than are the soluble solids.

From the data in Table I it is possible to estimate the number of oranges in any sample according to the degree of accuracy required for the mean values of the two factors. The probable error of the mean value for any random sample is given by the formula

$$\%\ \text{P.E.} = \pm \frac{2}{3} \frac{\text{S.D.}}{\sqrt{n}} \frac{100}{\text{M}} = \pm \frac{2}{3} \frac{\text{C.V.}}{\sqrt{n}}$$

where "n" is the number of oranges in the sample.

In the case of the soluble solids the probable error of a single sample will be ±6·4 per cent. of the reading. If, as in the present work, the sample contains say, twenty-five oranges, the probable error of the mean will be ± 1·3 per cent. of the value. Under these conditions the experimental errors, namely ± 0·1 per cent., are negligible compared with those due to sampling, so that the method is more than sufficiently accurate for the purpose. In the case of the acidity the probable errors for a single sample and for one of twenty-five oranges will be ±11·4 per cent. and ± 2·5 per cent. respectively. In order to reduce the probable error of sampling in the case of the acidity to a magnitude comparable with that of the soluble solids a sample of about sixty to seventy oranges would be required. For practical purposes, however, such a degree of accuracy would certainly not be necessary.

Relationship between the Soluble Solids and the Total Sugars.

In order to ascertain how far it is justifiable to employ the soluble solids as a direct measure of the sugars the relationship between these two factors has been studied. The curves of growth with time have been discussed for the soluble solids and sugars separately in a previous paper (2). It would appear that the two curves are not completely coincident, at least during the final ripening period. In this respect the results differ from those obtained in the case of grapes (5). With oranges, however, the curve for the soluble solids was applicable over a very limited range of concentration and any compounded curve could not be expected to express the relationship between these two factors over a more extended range. In the present case the data for studying this relationship have been obtained by determining the average values of the sugar content corresponding to each successive 0·5 per cent. range of the soluble solids. It has been shown (3) that the data from Trees 1 and 6 are abnormal and the results from these trees have been treated separately. The resulting data are shown in Fig. 1 and it is clearly seen that over a wide range of values the relationship is definitely linear. In order to ascertain whether this result is of general application data from other sources have also been studied. One set of data was obtained from some work by van Wijk (11) dealing with the sugar/acid ratio in oranges. Two further sets of data were obtained from work which had been carried out in previous years in connection with the effect of arsenate sprays upon the flavour of oranges (6). The one set, termed "L," was obtained from the same orchard in the Bathurst District from which the present more extended range of samples was obtained. The other set, termed "B.G.," was obtained from orchards in the Fort Beaufort District. These last oranges were characterized by a particularly high content of sugars and soluble solids and a low acidity. The relationship between the soluble solids and sugars in these cases is shown in Fig. 2 and is also clearly linear.

It is evident that these two factors are related by an equation of the form

$$\text{T.S.} = \text{m S.S.} + \text{C}$$

where "m" and "C" are constants.

In actual practice the value of "C" is negative. The constants have been calculated for each set of data and the values given in Table II. The calculated curves are shown in Figs. 1 and 2.

TABLE II.

Samples.	"m."	"C."	Average deviation.
Trees 2—5	0·83	1·16	±0·12
Trees 1 and 6	0·81	0·77	±0·28
"L"	0·81	1·24	±0·26
van Wijk	0·75	0·50	±0·28
"B.G."	1·09	3·62	±0·16

These results indicate clearly that it is justifiable to substitute the soluble solids for the sugar in studying the sugar/acid ratio, provided that due allowance be made for the corresponding increase in the absolute magnitude of the

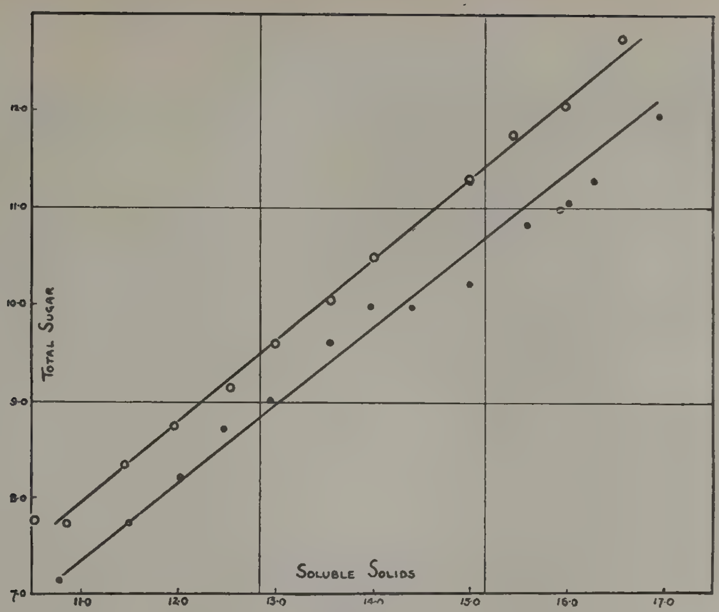

FIG. 1.

Relationship between Sugar and Soluble Solids.

○ Trees 2 − 5.

● Trees 1 + 6. 1·0 added to Soluble Solids.

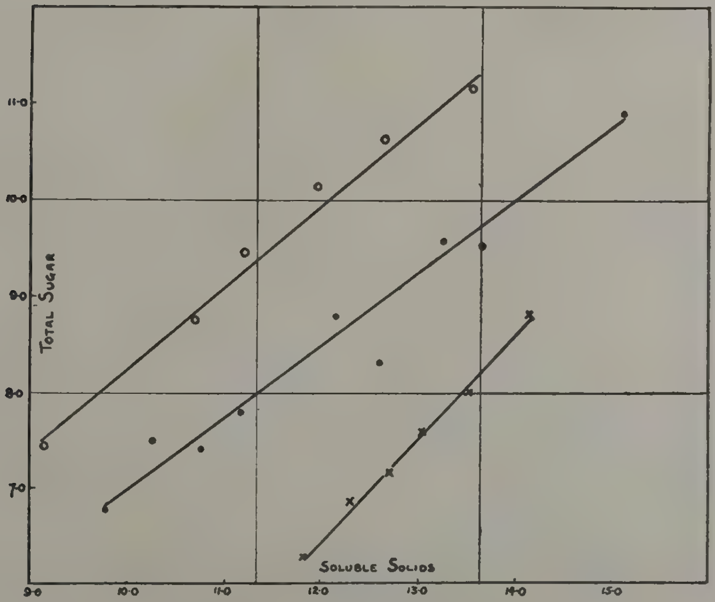

FIG. 2.

Relationship between Sugar and Soluble Solids.

○ Long. Sugar + 1·0.

● van Wijk.

× "B.G." {Sugar − 3·0.
Soluble Solids + 1·0.

resulting ratio. In this connection it is interesting to note that van Wijk (11) has derived the following approximation from his results.

$$\text{Sugar} = \text{Brix} - \text{Acid} - 1{\cdot}75$$

where Brix is the hydrometer reading.

This expression assumes that no correction is necessary to convert the Brix reading to a volume percentage. However, the range of values studied is somewhat limited, namely 10 to 14 Brix, so that any correction would be small and be included in the constant term. In addition the errors in the acidity due to sampling are relatively large and would serve as a further source of error in estimating the sugar in this way.

(*a*) *Significance of the Constants "m" and "C".*

In the case of the similar relationship for grapes (5) it was shown that the values of "m" and "C" were related to the growth constants for the two factors. It would, therefore, be expected that the above constants would be directly affected by the conditions of growth. From this point of view the results in Table II are distinctly interesting. It will be seen that the values of "m" for the first three sets are practically identical while the remaining two sets show distinct differences. It is significant that the values of "m" should vary in this way with the different localities from which the samples were drawn. In this respect the effect of environment appears to be greater in oranges than in grapes, but the differences may be accentuated in this latter case owing to the fact that the localities are more widely scattered. It must be concluded from the agreement between the first three sets that each locality is characterized by a given value of "m" which is independent of seasonal variations. These particular samples were obtained from the same orchard but gathered in different years. "m" must therefore be regarded as a constant dependent upon the internal factors of growth. It is a measure of the relative rates at which the sugar and soluble solids accumulate in the juice of the oranges. On the other hand "C" appears to be dependent upon the nutrient levels of the tissues, since high concentrations of sugar and solids are associated with high values of "C". Under such circumstances "C" would be directly affected by the conditions constituting the environment of the growing organism. This conclusion is evident from the variations in "C" for oranges from the same orchard at different times, and "C" must be regarded as a measure of the external factors of growth.

(*b*) *Errors due to the Relationship.*

It is clear that the sugar can be directly expressed in terms of the soluble solids, but the constants involved will depend upon the locality from which the samples are obtained. It is clear that for accurate results account would have to be taken of the variations in the constants caused by differences in locality, if it be desired to substitute the sugars by the soluble solids. If the average values be taken as representing the constants for a general equation, then

$$T.S. = 0{\cdot}86m - 1{\cdot}46.$$

In the case of the "B.G." samples, for example, the error involved in the change in the value for "m" would amount to about 20 per cent. of the value of the soluble solids and, therefore, very much greater than could be accounted for by sampling errors. The change in the value of "C" would introduce variations ranging from 0·22 to 2·16 in absolute magnitude, or from about 2·0 per cent. to 20·0 per cent. of the actual value. It is therefore clear that variations in "m" and "C", such as may be due to locality and seasons, cannot be entirely ignored when attempts are made to express directly the sugar in terms of the soluble solids. Such limitations must be borne in mind when the absolute magnitude of one factor is determined from the value for the other.

On the other hand the errors involved in any particular case are small and very much less than the errors of sampling. The probable error for the sugar content of a single orange due to random sampling will be ± 7·5 per cent. of the values. The average deviation of the values from the calculated data are given in the fourth column of Table II. In order to reduce the sampling error to this magnitude a sample of nine to ten oranges would be required.

COMMERCIAL PROCEDURE.

It is clear that it is justifiable to take the soluble solids as a measure of the sugars, provided that due allowance be made for the increase in magnitude of the resulting ratio. Owing to the difficulties connected with the direct determination of the sugar for rapid routine analyses and to the simplicity with which the soluble solids can be determined by means of a hydrometer the latter factor is always employed in commercial practice. The acidity of the juice is determined in the usual way by titration.

(*a*) *Determination of the Soluble Solids.*

In practice the soluble solids are determined by means of a Brix hydrometer which is graduated in terms of grams per 100 grams. The use of this instrument however, is subject to certain definite limitations on account of the increase in the experimental errors. In the first place it is impossible to read the instrument to less than 0·1 degree. In addition, especially in the case of a fruit-juice, the meniscus obscures the reading on the spindle so that, even under the best conditions there will be an error of ± 2·5 per cent. of the reading. Owing to the fact that the acidity is expressed in terms of grams per 100 c.c.

it is necessary to convert the Brix reading to a volume percentage, and a temperature correction is also frequently necessary, since the hydrometer is graduated for use at a standard temperature. For these purposes de Villiers (10) has published a table in which both corrections have been combined to give a single figure. Owing to the limitations in reading the instrument, however, an elaborate correction is hardly necessary. The simple corrections given in Table III should be sufficient for most practical purposes and will not introduce an error greater than ± 2·5 per cent. of the reading between the range 5-15° Brix.

TABLE III.

Temperature.	Correction.
15°—19° C.	$+\frac{1}{30}$ B.
20°—24° C.	$+\frac{1}{20}$ B.
25°—30° C.	$+\frac{1}{10}$ B.

The total experimental errors involved in the use of a Brix hydrometer under the above conditions will therefore amount to ± 3·5 per cent. of the reading. In these circumstances it is clear that the accuracy obtainable by the method does not lend itself to the use of an unduly large number of oranges in any given sample. With a sample of nine oranges the probable error due to sampling would be ± 2·2 per cent. which is considerably less than that due to the method as outlined above, namely, ± 3·5 per cent. of the value.

(*b*) *Temperature and Volume Corrections.*

Between limited ranges of temperature and concentrations the temperature correction is very nearly a linear function of the temperature. If the hydrometer be standardized at 20° C., the corrections may be obtained from the following:

Above 20° C. .. .. Correction = 0·063(" t " − 20)
Below 20° C. .. .. Correction = 0·047(20 − " t ")

These corrections will be applicable between the limits of 13° C. and 30° C. and a range of 5 to 15 per cent. soluble solids, and these ranges will cover practically all the conditions likely to be encountered in practice with oranges. The maximum error at the extreme limits of the ranges does not exceed − 0·04 and may be neglected in practice. It is clear that with a very close approximation the Brix reading may be corrected for temperature by the addition or subtraction of 0·05 for each degree above or below the standard temperature. The maximum error due to the use of such a correction will not exceed ± 1·0 per cent. of the reading. The importance of this simple correction will be clear when the Brix/acid ratio is studied.

In an earlier paper dealing with grapes (5) the Brix degrees were converted into soluble solids per 100 c.c. of juice by means of the formula

$$\text{S.S.} = \text{B} + 0{\cdot}0042\text{B}^2.$$

However, over a limited range it can be shown that the volume percentage of the soluble solids is practically a linear function of the Brix reading. In the present work with oranges the range of soluble solids was from 9 per cent. to 17 per cent. and this covered more than 95 per cent. of the total number of readings. For this range of soluble solids it was found that

$$\text{S.S.} = 1{\cdot}107\text{B} - 0{\cdot}68.$$

The maximum deviation was less than $-0{\cdot}04$ which again is negligible in comparison with the errors in reading the instrument. At the same time the maximum errors would tend to compensate the error due to the use of the unclarified juice.

RELATIONSHIP BETWEEN BRIX/ACID RATIO AND SUGAR/ACID RATIO.

From a practical point of view the direct use of the Brix reading in determining the relationship between the soluble solids and acidity would be of value, since such a procedure would obviate the necessity of applying corrections other than that for temperature. A knowledge of the relationship between the Brix/acid ratio and the sugar/acid ratio therefore is of practical importance. In the present work the Brix reading of the juice was obtained from the density of the juice by means of Tables. The average values of the sugar/acid ratio were determined for each successive 0·5 range in the value of the Brix/acid ratio. The relationship between these two quantities is shown in Figs. 3 and 4 and is clearly linear.

Hence

$$\frac{\text{T.S.}}{\text{A}} = \text{m}\frac{\text{B}}{\text{A}} - \text{C}$$

The constants were calculated for the same series of data as before and are given in Table IV.

TABLE IV.

Series.	"m."	"C."	Standard B/A.
Trees 2—5	0·88	0·73	5·7
Trees 1 and 6	0·87	0·70	5·8
"L"	0·886	1·15	6·1
van Wijk	0·80	0·51	6·0
"B.G."	0·92	0·89	5·6

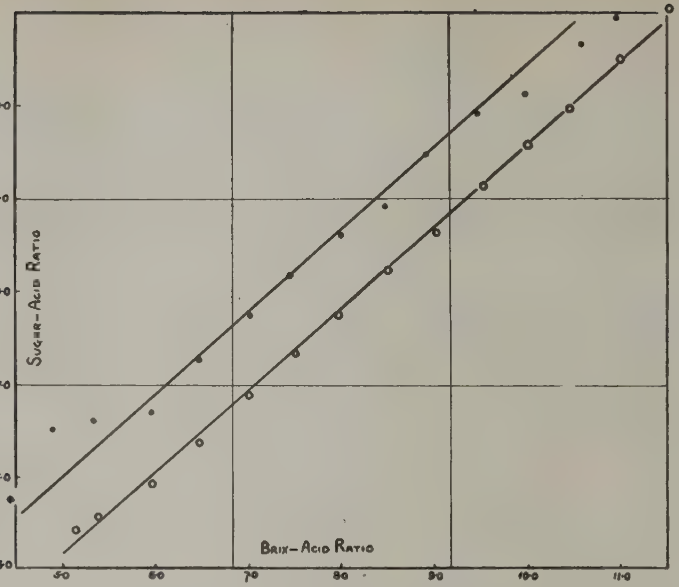

FIG. 3.

Relationship between Sugar-Acid Ratio and Brix-Acid Ratio.

○ Trees 2 – 5. Sugar-Acid Ratio + 0·5.

● Trees 1 + 6 { Sugar-Acid Ratio + 0·5. Brix-Acid Ratio – 1·0.

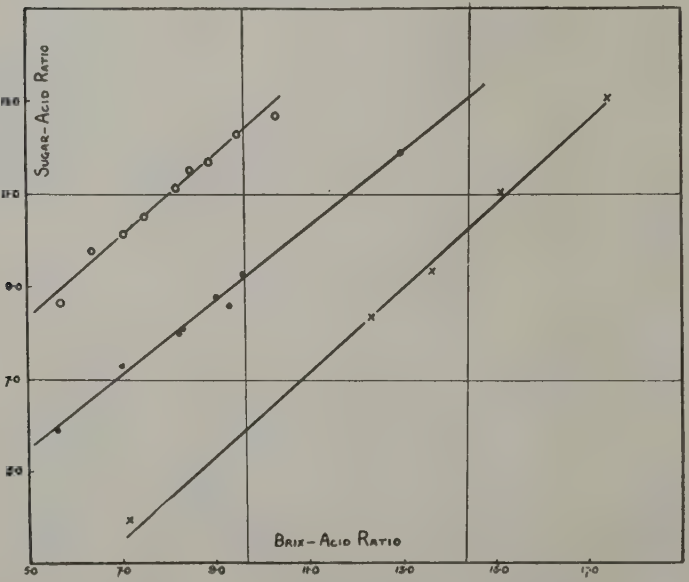

FIG. 4.

Relationship between Sugar-Acid Ratio and Brix-Acid Ratio.

○ Long. Sugar-Acid Ratio + 5·0.

● van Wijk. Sugar-Acid Ratio + 2·0.

× "B.G." Sugar-Acid Ratio – 2·0.

It is interesting to note that, although the variations in the value of "m" show the same tendency to vary with the locality as in the previous case, their range is reduced so that less error would be introduced by the adoption of a standard value for "m". The average value of "m" is 0·87 and the maximum error introduced by using this value would amount to about 9 per cent. instead of 20 per cent. as previously found. A similar conclusion can be drawn regarding the use of the average value of "C", namely 0·80. In this case the maximum error would be +0·35, equivalent to approximately 6·0 per cent. of the value for the Brix/acid ratio.

It is clear from the above results that the sugar/acid ratio can be directly calculated from the Brix/acid ratio without necessarily converting the Brix reading to a volume percentage, and this latter ratio would supply as much information concerning the quality of the fruit as either the solids/acid ratio or the sugar/acid ratio. The only correction required would be that for the temperature and a very simple method for this purpose has already been pointed out. In these circumstances a standard involving the direct Brix/acid ratio could be employed after making due allowance for the necessary change in the magnitude of the value. In the last column of Table IV the values for Brix/acid ratio corresponding to a value of 4·3 for the *sugar/acid ratio* and to a value of 6·0 for the soluble solids/acid ratio have been calculated for each set of samples. The results agree remarkably well and it is clear that the average value of 5·8 would serve as a suitable standard for the Brix/acid ratio for practical purposes. In practice the exact course of the changes leading up to the attainment of this equilibrium need not necessarily be defined. It is important that the value of 5·8 for the ratio must represent a definite equilibrium between the constituents of the fruit.

EFFECT OF SAMPLING ERRORS.

In employing a ratio such as this as an index of the degree of maturity and palatability of oranges it must be remembered that the value obtained is considerably influenced by the variations due to random sampling. It has already been pointed out that the co-efficients of variations for the soluble solids and acid are ±10·0 per cent. and ±17·0 per cent. respectively. It is clear that a knowledge of the probable error due to sampling is of considerable practical importance in interpreting the significance of the figure obtained for the ratio. For this purpose the mean values of the soluble solids/acid ratio, the standard deviations and the co-efficients of variation have been calculated for each weekly set of samples of the oranges employed during the investigation of the changes during ripening. The results are given in Table V.

The Table indicates clearly that the ratio increases as the fruit ripens, but the present results do not indicate the presence of any abrupt change to mark the initial stages of ripening. It must be concluded that the choice of a standard must be empirical and based upon a knowledge of the marketing requirements. The results indicate a tendency for the ratio to reach a limiting value at the end of the ripening period. This would be expected from the fact that both the sugar and acid content of the juice tend to reach limiting values at this stage.

TABLE V.

Time in Days.	Number in Sample.	Ratio.	Standard Deviation.	Co-eff. of Variation.
0	28	7·32	1·195	16·3
12	27	6·78	1·107	16·3
19	22	7·53	1·228	16·3
26	22	8·47	1·682	19·9
33	28	8·01	0·992	12·4
41	26	8·15	1·129	13·8
48	27	8·56	1·057	12·4
62	19	8·61	1·089	12·6
69	27	8·79	1·169	13·3
76	24	9·50	1·277	13·4
83	10	10·11	1·233	12·2
89	26	9·66	1·417	14·7
97	26	9·76	1·286	13·2
104	21	10·83	1·323	12·2
		Average	1·227	14·2
		"t"	0·70	2·75

On testing the changes in the absolute and relative variation with time by the "t" method it was found that the former remained practically constant during the whole period. In these circumstances it naturally follows that the relative variation must decrease with time. It may therefore be assumed that the average standard deviation will represent the normal value in any random sample of oranges. Similar conclusions can be shown to be applicable in the case of the sugar/acid ratio, but the average standard deviation in this case was found to be 1·058, which is in good agreement with the value $1{\cdot}227 \times 0{\cdot}875 = 1{\cdot}073$, deduced from the relationship between these two ratios.

THE PROBABLE ERROR OF THE DETERMINATION.

The percentage probable error of the mean value of the ratio of soluble solids/acids for a random sample of oranges will be given by

$$\%\text{P.E.} \quad = \quad \pm \frac{2}{3} \; \frac{\text{S.D.}}{\sqrt{\text{n}}} \; \frac{100}{\text{M}}$$

where "n" is the number of oranges in the sample.

In the general description of the methods employed in making the test (10) 500 c.c. of juice are suggested as being sufficient. In general, such an amount of juice will be obtained from about ten oranges and at the critical stage, where the value of the ratio is 6·0, the percentage probable error will be given by

$$\%\text{P.E.} = \pm\frac{2}{3}\ \frac{1\cdot227}{\sqrt{10}}\ \frac{100}{6} = \pm\ 4\cdot3\%.$$

Under these conditions the value may be within the range 5·25-6·75 without loss of significance solely on account of sampling errors. If, however, a Brix hydrometer be used to determine the soluble solids, the experimental errors of the reading must be taken into account. These may vary from 2·5 per cent. to 3·5 per cent. depending upon the nature of the corrections made to the readings. In practice, therefore, the ratio may well lie within the limits 5·0-7·0 without loss of significance. It is quite clear that the variations due to sampling are sufficiently great to destroy any advantage that might be gained by the use of refined methods of testing the juice for practical purposes. It is suggested that the Brix/acid ratio be used directly and the only correction necessary will be the temperature correction which may be simplified by adding or subtracting 0·05 for each degree above or below the standard temperature. More refined methods than this of estimating the soluble solids are wholly unnecessary in assessing the quality of oranges under the present conditions. The value of the Brix/acid, corresponding to the present value of 6·0 for the soluble solids/acids ratio, would be 5·8.

Alternative Procedure.

The results indicate that these ratios do not fulfil satisfactorily the requirements as a critical measure for assessing the quality of a sample of oranges, and the possibilities of improving the test must be examined. Since the quality of the fruit is so greatly dependent upon the sugar and acid content of the juice attention is naturally focussed upon the use of either of these two factors as standards of reference.

(*a*) *Sugar Content.*

In the first place the direct estimation of the sugar content of the juice does not lend itself to adaptation for routine testing in practice, and for this reason the soluble solids as measured by a hydrometer, such as the Brix, must be substituted as a measure of the sugar content. Such a step, however, immediately introduces a comparatively large experimental error into the method of determination and immediately limits the number of oranges which can be profitably used to form a composite sample. In addition, however, when the orange has reached a stage when it is suitable for consumption, the concentration of soluble solids does not show any rapid changes, and the subsequent increase during ripening is relatively small. These conditions, therefore, tend to impose very severe limitations on any information to be gained from the soluble solid content of the juice. The idea of using a Brix reading of the juice by itself may be abandoned as an exact standard for assessing the quality of oranges.

(*b*) *Acid Content of the Juice.*

Examination of the changes in the sugar/acid ratio indicate that the magnitude of the ratio depends very greatly upon the acid content of the juice. Comparatively small changes in the acidity are accompanied by relatively large changes in the value of the ratio. In the early stages of the final ripening period the relative changes in the acidity are very much more rapid than in the sugar content, so that the change in the flavour of the fruit must be closely related to the changes in the acid content at this stage. These facts indicate that attention should be mainly focussed on the acidity of the fruit as a criterion of quality at this stage, which, in practice, is the most important.

In dealing with the effect of wilting upon the sugar/acid ratio it has been pointed out (7) that oranges from "sweet" trees had a *lower* sugar content than oranges from "sour" trees but that the acidity in the former case was also *lower* so that the ratio was nevertheless *high*. de Villiers (10) states that soluble solids/acid ratios higher than 6·0 are regarded as indicating a sweet fruit, particularly when the acidity is normal or high. When a high ratio is due merely to a *low* acidity the flavour tends to be insipid. The importance of the acid concentration is therefore obvious and it is concluded that in the great majority of cases a knowledge of the acidity will provide all the required information. Under the present conditions of testing oranges for maturity, a determination of acidity is part of the normal procedure, and by adapting a standard based on the acidity, the necessity for reading the Brix degrees and calculating the ratio is obviated. The maximum concentration consistent with the accepted standards of quality for marketable fruit must, of course, be defined by experience, but from the results obtained during the present investigation it is suggested that a value of 2·0 per cent. be regarded as the maximum limit of acidity for export fruit.

The acidity can be determined with an experimental error of ± 0·4 per cent. of its value so that the errors will be almost entirely confined to those due to sampling. With a sample of ten oranges the percentage probable error of the mean will be given by

$$\%\text{P.E.} = \pm \frac{2}{3} \; \frac{17 \cdot 1}{\sqrt{10}} = \pm 3 \cdot 6\%.$$

The corresponding error in the case of the Brix/acid ratio was ± 4·6 per cent. and the error in the case of the acidity can easily be reduced by increasing the number of oranges in the sample. In this respect the use of the acidity alone has a decided advantage over the soluble solids/acid ratio. In addition the acidity forms a direct measure and is capable of simple interpretation.

SUMMARY.

During an investigation into the changes in the composition of oranges during ripening a large number of observations of the soluble solids, the sugar and the acid content of the juice was obtained and these provide data for a consideration of the various factors which affect the determination of the sugar/acid ratio in commercial practice.

The experimental errors of a single determination of the sugar or acid content were of the same order, namely, ± 0·3-0·4 per cent. of the reading. The soluble solids were calculated from the density of the juice and the probable error of a single determination was ± 0·1 per cent. and failure to clarify the juice completely did not introduce a greater error than ± 1·0 per cent. of the value.

The probable errors of sampling were relatively large. The absolute and relative range of variation of the soluble solids did not change significantly during ripening and the average values were 1·276 and 9·6 per cent. respectively. In the case of the acidity the standard deviation decreased with time, but the co-efficient of variation remained more or less constant, namely, ± 17·1 per cent. This might have been expected from the extent of the changes in these two factors during ripening.

It was shown that the relationship between the sugars and soluble solids in the juice was linear over a wide range so that the latter can justifiably be used as a measure of the sugar content of the juice. The constants, however, are dependent upon the internal and external factors of growth.

In commercial practice, however, the soluble solids are generally estimated by means of a Brix hydrometer and the experimental errors are increased to ± 2·5 per cent. of the reading. Volume and temperature corrections are also necessary. The temperature correction can be made by adding or subtracting 0·05 for each degree above or below the standard temperature. The maximum error in this case will not exceed ± 1·0 per cent. of the reading.

It was shown that the sugar/acid ratio was a linear function of the Brix/acid ratio so that the latter ratio can be conveniently substituted for the former without the necessity of converting the Brix reading to a volume percentage. The value of 5·8 for the Brix/acid ratio was found to correspond to a value of 6·0 for the soluble solids/acid ratio.

The range of variation as measured by the standard deviation did not show any significant change in magnitude during ripening and the average value was ± 1·227. It was found that in practice, with a sample of ten oranges, the soluble solids/acid ratio might lie within the range 5·0-7·0 without loss of significance. The somewhat large errors due to sampling destroy any advantages that might be gained by using refined methods for such practical purposes.

It is pointed out that the sugar or the soluble solid content alone does not provide a suitable basis for judging the quality of the fruit and it is suggested that, since the acidity at the critical stage must be closely related to the flavour of the fruit, the acid content of the juice by itself will provide practically all the required information. The acidity can be determined with great accuracy and the range of significance can be limited by modifying the number of oranges in the sample. It is suggested that the maximum limit of acidity be taken as 2·0 per cent.

In conclusion the author would like to thank Dr. B. de C. Marchand for the interest he has taken in the preparation of this paper.

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SCION INFLUENCE IN CITRUS*

By F. F. HALMA

University of California, Citrus Experiment Station, Riverside, California

THE commercial Citrus varieties in California are practically all propagated by budding on sweet orange, sour orange or grapefruit. All three rootstocks are distinct species. Thus, a budded citrus tree is a symbiotic unit which may be composed of two varieties; for example, Valencia orange on sweet orange; or, it may be composed of two species; as Eureka lemon on any of the three commercial stocks. *Poncirus trifoliata,* although of no importance as a rootstock in California, is still considered the outstanding stock for the satsuma orange in which case stock and scion are not only of a different genus, but the former is deciduous and the latter evergreen.

Scion and stock effect has probably been studied more extensively in apples than in any other crop. Hatton (3) and his associates have attacked the problem largely from the standpoint of rootstock effect on scion, while Swarbrick (4) has focussed attention on scion influence on stock. Both investigators, however, are fully aware of the existence of an inter-relationship between the two parts of the tree. Rootstock effect on scion in citrus has been studied by Webber (7), who has also summarized the known scion influences, hence they need not be reviewed here. The object of this paper is to report additional evidence on scion influences in citrus recently observed by the writer, which are believed to be of special interest in that they indicate a relationship to method of propagation. These observations were made incidentally on trees grown for other purposes and are, therefore, limited in scope.

It is fairly well known that the general form of the root system of mature citrus trees in California is characteristic of the rootstock variety. Thus, irrespective of the scion variety, the sweet orange and grapefruit stock have a predominantly lateral root system, while the sour orange has a deep, penetrating taproot system. For example, in one Eureka lemon orchard, the average number per tree of main roots of sixty-four trees on sour orange stock was fifteen, of which 65 per cent. were typical taproots, whereas the root systems of a similar number of trees on sweet orange stock were practically devoid of taproots. It should be mentioned in this connection, as pointed out by Webber (7), that among citrus seedlings, especially sour, there occur "variants" which differ from the standard type in habit of growth, character of foliage, lack of oil glands in leaves, etc. It is therefore conceivable that their root systems may also vary from the standard type. The lateral root system of the sweet orange and grapefruit, however, is the result of a gradual change from an original taproot in the early seedling stage (Fig. 1). Since the method of propagation will be referred to later as being a factor in scion influence, it should be mentioned in this connection that the ordinary budding method requires seedling rootstocks from one and one-half to two and one-half years of age, which means that the root system is at least two years old, and therefore well established before the tops of the scion variety begin to function.

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* Paper No. 286, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

A similar lack of scion influence on the form of root system was observed on a limited number of trees having rootstocks propagated by either twig or root cuttings (Halma, 1). This is of special interest because of the fact that the original root system of cuttings is entirely lateral, and continues to remain lateral for all commercial varieties except the sour orange which, after it is transplanted to the nursery, develops a taproot system quite like a seedling (Figs. 2, 3, 4).

Apparently normal citrus trees can also be produced by twig grafting (Halma, 1). This is accomplished by tongue-grafting together two leafy twigs representing the desired stock and scion varieties, and then rooting them in a propagating frame (Fig. 5). Like ordinary cuttings, the original root system is lateral, but it was noticed in transplanting a few trees to the orchard three years later, that the sour orange stock when combined with the Eureka lemon had failed to develop its characteristic taproot system. The root system of the reverse combination was also lateral, but this was to be expected since the Eureka lemon root system is of that type (Fig. 6). The same situation was observed with several trees planted a year later. The trees on sour stock four years after planting in the orchard still retain their original lateral root system with no sign of taproot development. Apparently, then, the Eureka lemon scion effected a radical change in the root system of the sour orange, whereas, in the reverse combination, the sour orange scion was unable to impose its type of root system upon the Eureka lemon.

This difference in scion effect was also observed when the two varieties were combined by root grafting. The method was the same as that used for twig grafts except that instead of a leafy twig, a root piece seven to ten centimetres long and about one centimetre in diameter served as the rootstock (Fig. 7). Twelve plants of each combination were grown in the nursery for three years before they were examined. As seen from Figs. 8 and 9 the lemon scion inhibited taproot development in the sour orange stock; whereas, in its turn, the lemon root system was not affected, with the exception of two cases which should be considered doubtful since they showed a slight indication of taproot development.

The consistent behaviour of these two combinations, whether twig or root grafted, suggests that scion effect on form of root system depends on at least two factors. Apparently the absence of roots prior to grafting is one of these; for, as has been stated, the Eureka lemon scion effect is absent in budded trees. The other factor is the scion variety which does not necessarily mean, however, that the Eureka lemon exerts a similar influence on other varieties. Unfortunately, a study of scion influence on the form of root system is somewhat limited owing to the fact that the sour orange is the only commercial variety so far tested that produces a taproot system when grown from cuttings. Among the lesser known varieties which develop a similar root system is the Rusk Citrange, a cross between sweet orange and *Poncirus trifoliata*, which might be suitable experimental material. But it would be possible by the twig-graft method to test various combinations with the sour orange, serving either as scion or stock. Such studies should show whether scion influence on root system is governed by differences in rapidity with which varieties take root when grown from cuttings. As shown by Halma (1), the Eureka lemon, for example, roots more rapidly than the sour orange, and this might be the reason the former dominates the latter when grafted together. But, were this the case, then the sour orange should dominate the sweet orange, since the former roots more rapidly than the latter, a point which could easily be cleared up with twig grafts. In this connection it should also be mentioned that twig grafts grown in water culture should furnish excellent material for a study of scion influence on root anatomy as well as on fibrous roots. Observations of cuttings in water culture show that the fibrous roots of the lemon can be readily distinguished from those of the sour orange by their texture, and any scion influence would be easily discernible.

The complexity of the problem of scion influence on form of root system is also apparent in apples. According to Swarbrick and Roberts (5), the form of root system of apple trees propagated by grafting on to root pieces is determined by the scion variety, but this scion influence is lacking when propagated by budding. They believe that lack of scion influence in the latter case is due to the intervening piece of stem between scion and root proper, for, if budded at or near the crown root, the scion variety again determines the root character of the stock. Vyvyan (6), on the other hand, found that the distinctive characters of the root systems of two-year-old No. II and No. VI stocks remained approximately constant whether unworked, worked with scions of their own variety, or with Beauty of Bath or Grenadier and whether the latter were root-grafted on to piece roots or stem grafted. But he points out that the scion and stock varieties, and age of the trees observed by him, were not the same as those studied by Swarbrick and Roberts.

The contradictory behaviour of apple root grafts seems to be analogous to that of citrus root grafts where the lemon scion changed the form of root system of the sour stocks, but, as a stock, was not influenced by the sour orange scion. Obviously the theory of Swarbrick and Roberts to the effect that the lack of scion influence in stem budded apple trees is due to the presence of a piece of stem between the bud union and root proper does not apply to citrus, since both root and stem grafts behaved alike. Furthermore, if the "stem effect" were a factor in citrus, then the Eureka lemon scion effect on sour orange root system should be the same, whether the combination is effected by the twig-grafting or by the budding method, since in both cases a piece of stem intervenes between the graft or bud union and the root proper. But this is not the case. The presence or absence of a root system prior to grafting or budding is apparently not considered a factor in apple scion influence by either Swarbrick and Roberts, or Vyvyan, since they compare the behaviour of root grafts with that of trees propagated by budding on to previously rooted stock.

The predominate lemon scion influence was found to manifest itself in another manner when the root grafts were subjected to a test developed by Halma and Haas (2) for identifying the rootstock variety. Before giving results it may be desirable to give the method used as it has been modified since it was first published.

> Enough bark is lifted from the rootstock trunk to make at least 0·5 grams when dried at about 70° C. After drying it is ground fine enough in a mortar to pass through a 40-mesh sieve. 0·5 grams of this powder is placed in a beaker and 20 cc. of distilled water is added. After several minutes the contents of the beaker are transferred to filter paper and the residue washed with 10 cc. of water. The 0·5 grams of powder are therefore leached with 30 cc. of water, and 5 cc. of the filtrate are made alkaline with two drops of potassium hydroxide prior to adding 10 drops of a saturated solution of copper sulphate. This is followed by 10 drops of mercury reagent which is made up as follows: 160 grams of re-distilled mercury are dissolved in 160 grams of red fuming nitric acid (specific gravity 1·60) and this is finally diluted with 320 cc. of distilled water. 10 drops of this reagent are added to the contents of the test tube and boiled. The resulting colour of the solution identifies the variety. Thus the sour orange bark extract assumes a sort of amber colour and that of the sweet orange a dark pink. The Eureka lemon, although not ordinarily used as a stock, can easily be distinguished from the sour orange by a reddish colour reaction. These two varieties can be further separated by adding two drops of ferric chloride solution to 5 cc. of bark extract. In this test the Eureka lemon extract turns a blackish brown and the sour orange a much lighter brown. (Tests with Rough lemon bark extract shows that its colour reaction is distinctly different from that of the Eureka lemon although both varieties are classified as *Citrus limonia*.) Although the nature of the colour reactions has not been investigated it has been found that with budded trees it is not

influenced by age of tree, time of year at which the bark sample is taken, and most important of all, the substance involved in these tests apparently does not move across the bud union.

Applying this tests to the two root-grafted combinations, it was found in both cases that the bark extract of the scion and that of the original root piece upon which it was grafted gave a colour reaction typical of the variety, with two doubtful exceptions of two sour root pieces out of the twelve which seemed to indicate a slight lemon influence. But bark extract of the roots proper of the sour orange stock, developed after grafting, gave a colour reaction which was neither that of the lemon nor sour orange, although much closer to the former. The roots proper of the lemon stock, on the other hand, were apparently not affected by the sour orange scion. Here again, as in its influence on the form of root system, the Eureka lemon scion exerted an influence while in turn it was not affected as a stock.

Finally it should be mentioned that there are indications of scion influences on external colour of the main roots. Normally the colour of the root of sour orange is light and that of Eureka lemon has a reddish tinge. (The colour of the roots of the latter has not been observed on roots of the Rough lemon.) In the freshly dug trees, it was quite noticeable that the colour of the roots of both varieties was affected to an extent that they resembled the colour of the roots of the scion variety more closely than that of their own. This observation is of interest in that it indicates also a sour orange scion influence which did not manifest itself in the form of root system or in colour reaction of root bark extract.

SUMMARY.

Eureka lemon scion grafted on to twigs or root pieces of sour orange effected a change in the inherent form of the root system of the latter ; it modified the colour reaction of the root-bark extract and also the external colour of the roots. The effect of the sour orange scion on the Eureka lemon stock was practically limited to a modification in external colour of the roots. The method of propagation is assumed to be a factor, since these scion influences have not been observed to occur in budded trees.

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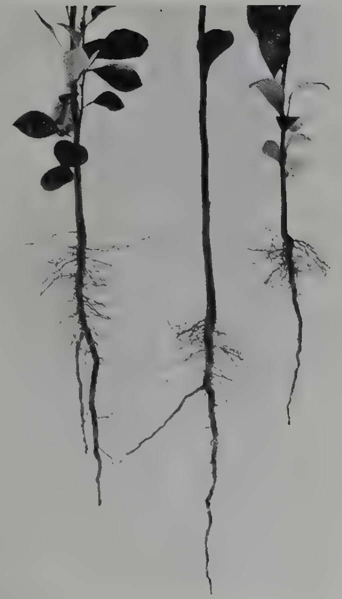

Fig. 1.

Taproot system of Citrus seedling.

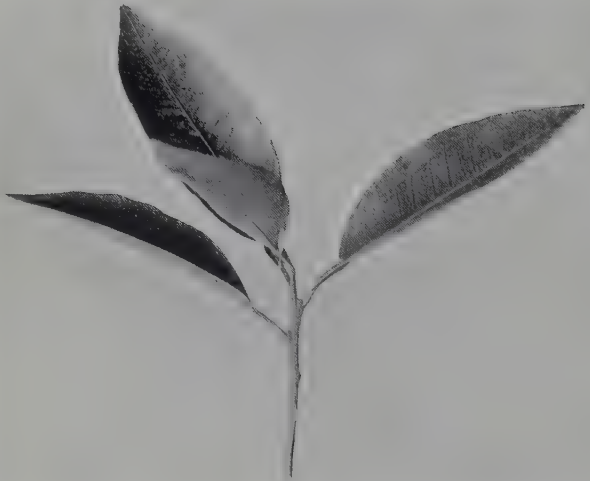

Fig. 2.

Type of cutting used in propagation.

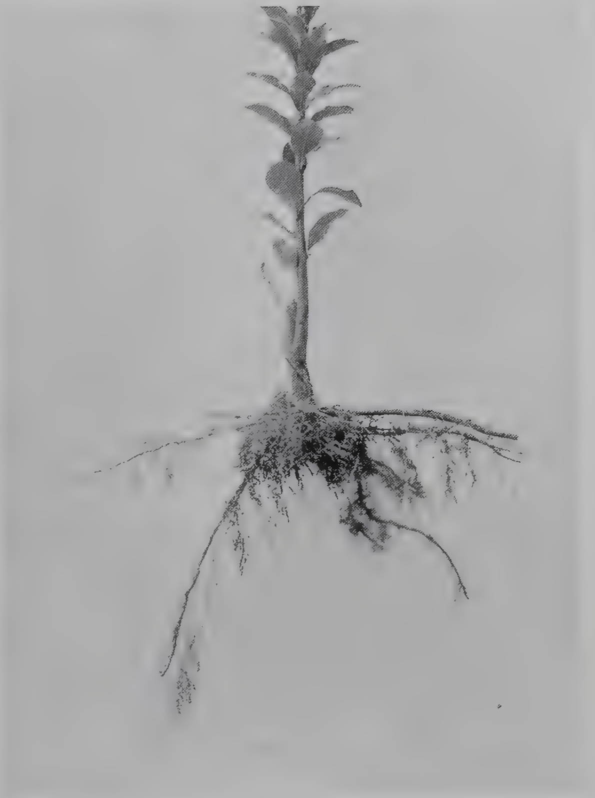

Fig. 3.

Three-year-old Lemon cutting, showing a lateral form of root system typical of other standard varieties.

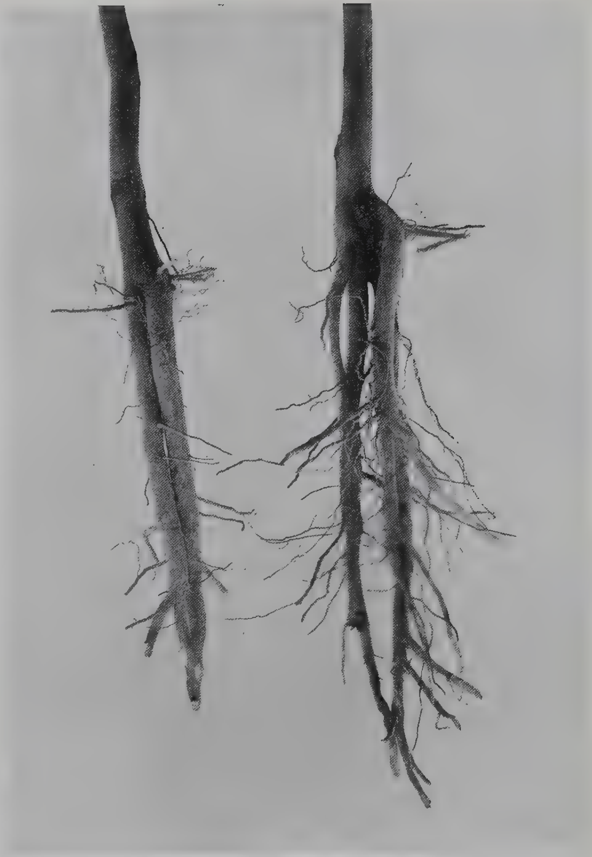

Fig. 4.

Three-year-old sour orange cuttings showing a taproot system typical of this variety.

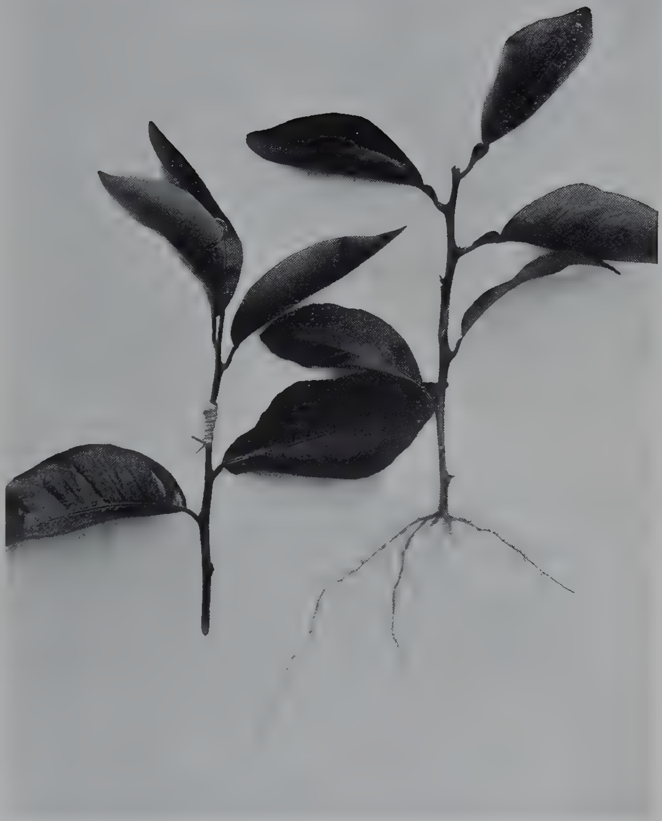

FIG. 5.
Twig grafts before and after rooting.

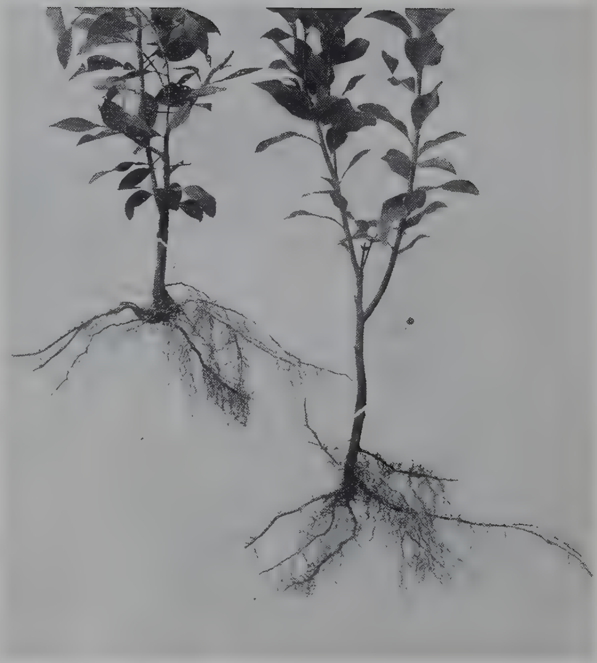

FIG. 6.
Two-year-old twig grafts showing lack of taproots in sour orange stock when combined with Eureka lemon scion.

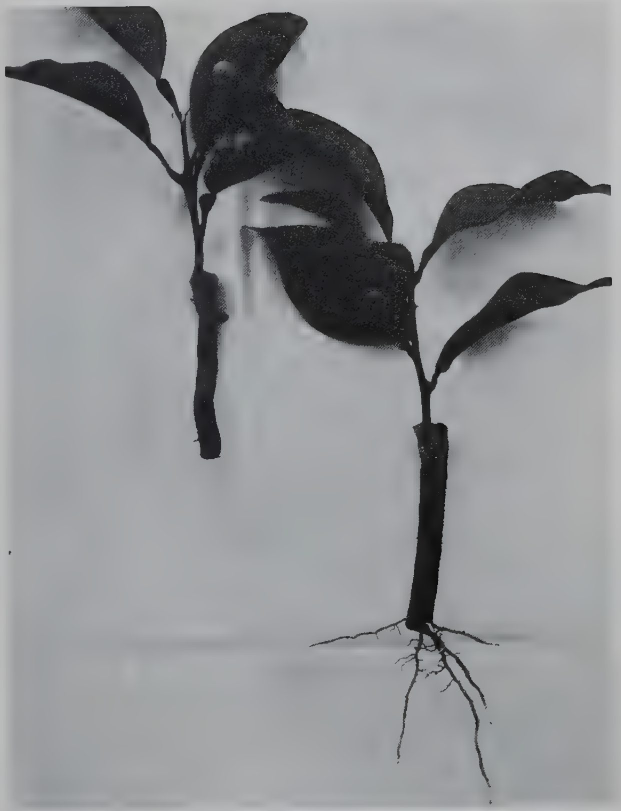

FIG. 7.
Root grafts before and after rooting.

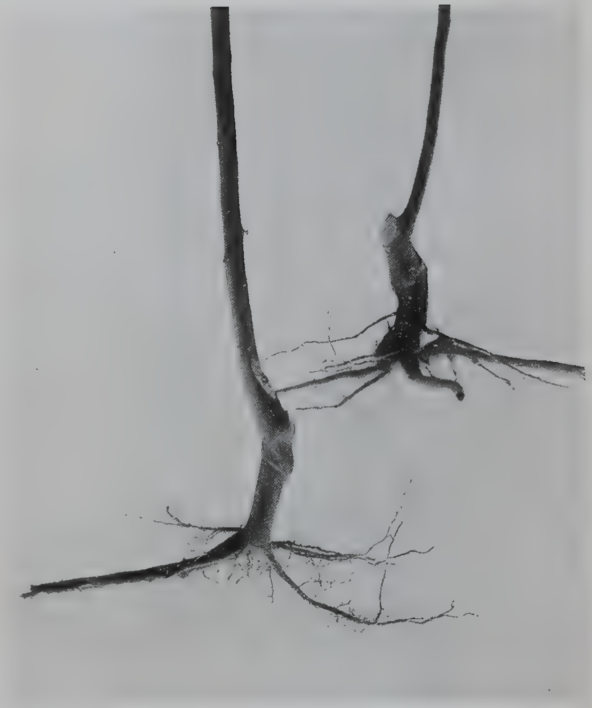

FIG. 8.
Three-year-old root grafts showing lack of taproots in sour orange stock when combined with Eureka lemon scion.

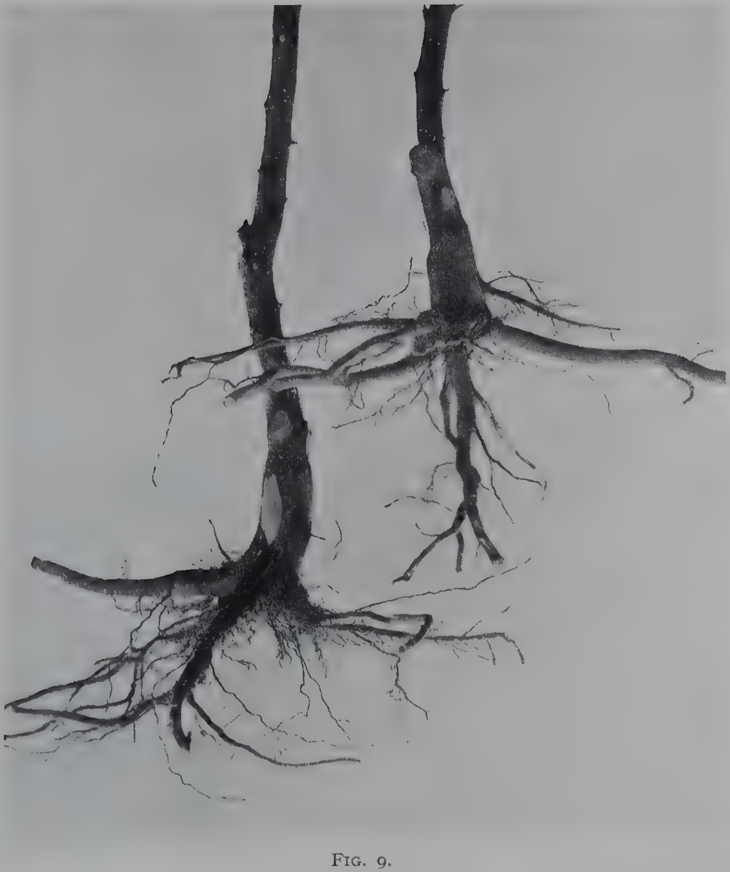

FIG. 9.

Three-year-old root grafts; on left showing lack of sour orange scion influence on Eureka lemon root system; on right, a doubtful effect.

NOTES ON THE WITHER-TIP DISEASE OF PLUMS CAUSED BY *SCLEROTINIA CINEREA* AND ON THE BLACKENING OF APPLES CAUSED BY *SCLEROTINIA FRUCTIGENA*

By R. DRUMMOND
Botany School, Cambridge

A. WITHER-TIP DISEASE.

SINCE there is little experimental evidence to indicate in what manner young plum shoots become infected with *Sclerotinia cinerea*, an attempt was made in the experiments described later to bring about infection of such shoots by various methods.

Nearly all previous work on this subject has been carried out by Wormald. In 1918, he conducted a series of experiments on growing shoots in an orchard and in a greenhouse, placing conidia on uninjured leaf laminae, on the glands at the top of the petioles and also on punctures made through the leaves. In no case could any difference be detected between the inoculated leaves and the controls; it appeared probable, therefore, that the leaves must receive some special form of injury before the fungus could invade the tissues. Wormald suggested that injury by aphides might possibly provide the necessary conditions for infection.

Four series of experiments were carried out by the writer at Cambridge during the Spring and Summer of 1933, the method of inoculation being similar in each case. A pure culture of *Sclerotinia cinerea* (forma *pruni*) was obtained and the conidia used for inoculation were formed on sterilized potato slices in tubes. The pustules of conidia were transferred to sterile water in test tubes and were shaken up to form a spore suspension. A drop of this liquid was transferred with a sterile brush or inoculating needle to the leaf laminae or petiolar glands, etc. Attempts were made to infect young leaf laminae, nodes, glands and the bark of young shoots.

In some of these experiments, the portion of the shoot inoculated was punctured previously with a needle to simulate insect attack; in others no wounding took place. After inoculation the shoots on the trees were enclosed in paper bags, which were moistened inside with water before being tied in position. The cut shoots were kept in water in a greenhouse, the basal ends being re-cut frequently in order to prevent the vessels from being choked with gum. Some cut shoots were covered with belljars; others were exposed to the air.

The shoots were examined daily and notes were made of successful infections and of the rate of spread of the brown discoloration.

EXPERIMENT 1. INOCULATION OF PLUM TREES IN POTS IN A GREENHOUSE.

Twenty inoculations were carried out on Victoria plum shoots between May 1st and May 22nd, ten being carried out through punctures of the tissues and ten without punctures. Punctured bark of young stems, petiolar glands, nodes and young leaf laminae invariably gave positive results, brown discoloration of the infected parts caused by *Sclerotinia cinerea* being visible in from one to two weeks. The spread of the discoloration generally ceased abruptly at from four to five weeks after inoculation. No inoculations of mature punctured leaf laminae were successful.

Out of the ten inoculations of unpunctured leaf laminae, glands, nodes and young stems only one was successful, and since this may have been due to some slight injury of the tissues caused by the process of inoculation, the evidence of infection without wounding was not conclusive. The trees used were not infested with aphides.

EXPERIMENT 2. INOCULATION OF PLUM TREES IN THE OPEN.

In a second experiment twenty inoculations were carried out in May on Cox's Emperor, Victoria, Early Rivers and Czar plum trees in the open. Only three infections were obtained: one of an unpunctured leaf lamina, one of an unpunctured petiolar gland and one of a punctured leaf lamina. The small measure of success in this experiment was probably due to the prevailing hot, dry weather. Wither-tip is most abundant during a wet season. The trees used in this experiment were infested with aphides.

EXPERIMENT 3. INOCULATION OF PLUM SHOOTS CUT FROM TREES IN THE OPEN AND KEPT IN WATER IN A GREENHOUSE.

Twelve out of eighteen of these inoculations, also carried out in May, were successful. Eight glands (including five unwounded) and four young leaf laminae (including two unwounded) were infected. Mature leaf laminae were never infected.

The mycelium generally grew so rapidly that the infected shoots were completely killed, but a clearly marked line occasionally separated diseased from healthy tissues. Conidial pustules were frequently formed on these shoots even when they were exposed to the air.

Since these shoots were taken from trees in the open, they had all undergone the risk of insect wounding and many were actually infested with aphides. The results of this experiment agree with those of Wormald (1922) when he was working with Shoot-wilt of plum.

EXPERIMENT 4. INOCULATION OF PLUM SHOOTS CUT FROM TREES IN A GREENHOUSE AND KEPT IN WATER.

A fourth experiment was carried out in July on shoots which had not been infested with aphides, cut from Victoria plum trees growing in a greenhouse. Six inoculations of petiolar glands resulted in two out of four unpunctured glands being infected while the two punctured ones were readily attacked.

GENERAL OBSERVATIONS.

In the successful infections of shoots on trees the effects of the fungus were first visible nine days after inoculation, while on cut shoots infection was apparent in from two to five days. The fungus passed rapidly from the leaf lamina or gland down the petiole to the stem and attacked the lower petioles and leaves as it extended downwards. When lower leaves were inoculated the fungus passed both up and down the stem. On trees the brown discoloration extended four or five inches down the shoot and generally ceased abruptly near a node. On cut shoots the fungus grew so rapidly that it often passed into the previous year's wood. The rate of spread of the discoloration from the time it was first visible until it stopped, varied from 1¼-1½ inches per week on the attached shoots to from 3-4 inches per week on the cut shoots.

B. BLACKENING OF APPLES.

Since there is a certain diversity of opinion as to whether the Blackening of stored apples caused by *Sclerotinia fructigena* is due to a particular strain of that fungus or is merely dependent upon the maturity of the fruits, observations were made on apples inoculated with strains of the fungus which had been isolated from typical brown and black rotted specimens.

Spinks (1915) and (1916) indicated that apples of the same variety showed the same type of rot no matter whether the strain of fungus used had been isolated from a brown or from a black rotted apple. It appeared that the type of rot produced by the fungus depended entirely upon the condition of the apple.

A different result was obtained by Wormald (1919), who concluded that the Blackening was not invariably due to the maturity of the fruit but might be produced by a particular strain of *Sclerotinia fructigena* which could only be isolated from black rotted apples.

The work of Spinks (1916) showed that a maturity factor was involved. Apples inoculated later in the season blackened more readily than those inoculated in the early Summer. Wiltshire (1919) found that the tendency to black rot was intensified as the season advanced.

Pure cultures of *Sclerotinia fructigena* were isolated by the writer at Cambridge early in 1933 from typical specimens of black and brown rotted apples. Since it was suspected that the age of the fruit influenced the formation of the Blackening, two methods of eliminating differences of maturity between apples used in the same experiment were tried. In the first method, apples were inoculated on both sides: on one side with the fungus from the black apple and on the other with the fungus from the brown apple. In the second method apples were surface sterilized and cut in two with a sterile scalpel; one half was then inoculated with the fungus from the black rot and the other half with the fungus from the brown rot.

The first method gave unsatisfactory results, since three weeks after inoculation it was difficult to distinguish the parts occupied by the two inocula. On March 14th three apples (two Jonathans and one Newtown pippin) were inoculated by the second method. Each half was placed in a Petri dish lid moistened with sterile water, halves of the same apples being marked similarly. After inoculation the apples were left under belljars, arranged so as to allow a current of air to circulate, and were examined after two weeks and again after five weeks.

The effects of the two isolations of *Sclerotinia fructigena* were identical since opposite halves of the same apples were similar in colour and appearance whether inoculated with the fungus from the black apple or from the brown apple. All showed Blackening after ten days, the skin being smooth and rubbery. No further changes were observed four weeks later. Young apples inoculated in July by a similar method became light brown and many buff-coloured conidial pustules were produced. There was never any sign of nigrescence.

On the other hand, apples inoculated with a strain of *Sclerotinia cinerea* isolated by the writer from Morello cherry blossoms last Summer blackened irrespective of either age or season. This fungus agreed in culture with the description of *Sclerotinia cinerea* (forma *Mali*).

The results of these experiments strengthen the opinion that old age in the apples favours the development of the Blackening caused by *Sclerotinia fructigena*.

SUMMARY.

A. WITHER-TIP DISEASE OF PLUMS (*Sclerotinia cinerea*).

1. Inoculations of shoots on plum trees (free from aphides) in a greenhouse were not successful unless the tissues had been punctured.

2. Few inoculations of shoots on plum trees in the open were successful, probably on account of the prevailing dry weather. One inoculation of an unpunctured leaf lamina, one of an unpunctured petiolar nectary and one of

a punctured leaf lamina gave positive infections. These shoots had been attacked by aphides.

3. Inoculations of punctured and unpunctured cut shoots kept in a greenhouse were successful even when the shoots had not been previously infected with aphides.

4. Young leaf laminae and petiolar glands were the parts of the shoots most readily infected in these experiments. Mature leaf laminae never became infected.

B. Blackening of Apples (*Sclerotinia fructigena*).

1. Blackening of old apples was produced equally well by *Sclerotinia fructigena* whether isolated from black or from brown rotted apples.

2. Blackening of both old and young apples was produced by *Sclerotinia cinerea* isolated from Morello cherry blossoms.

ACKNOWLEDGMENT.

I wish to thank Mr. F. T. Brooks for his guidance in this work.

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ROOT STUDIES

V. ROOTSTOCK AND SOIL EFFECT ON APPLE ROOT SYSTEMS

By **W. S. ROGERS** and **M. C. VYVYAN**

I. INTRODUCTION.

KNOWLEDGE of the root systems and root development of fruit trees is not yet in a very advanced state. Little detailed research has been carried out on this subject. Nevertheless the importance of the root system is evident, for it is known that through the roots the tree absorbs its water and mineral salts. In fact, everything of which the branches, trunk, leaves, fruit and roots of the tree are composed is normally absorbed through the roots, with the exception of the element Carbon, which is taken up by the leaves by photosynthesis.

It is therefore of primary importance to the grower and to the scientist to know where the roots of his various plants are and how they react to differences of soil texture, moisture, nutrient and other conditions. To the fruitgrower especially is this knowledge important, for he is accustomed to graft or bud his varieties of fruit tree on to a number of different rootstocks, each of which may produce a definite type of root system, through which the behaviour of the tree is very considerably modified. It is to the grower's advantage to use to the uttermost this power of controlling the tree by selection of the rootstock, and to suit his root system to the existing conditions or modify the conditions to suit the root system so as to obtain the result he requires.

The authors do not propose to give an exhaustive survey of previous work, since a detailed memorandum on research work on roots is at present being prepared by the Imperial Bureau of Fruit Production.

Recent publications on the effect of various rootstocks are: "The Influence of different rootstocks upon the vigour and productivity of the variety budded or grafted thereon" by R. G. Hatton (2); and "Stock-Scion relationships" by R. G. Hatton (3).

These deal mainly with stock effect as shown in top growth and fruiting, but some details are given of the differences in root hold shown by resistance of tree to blowing over and of the development of fibrous root in young stocks, as found when the young trees were dug up.

A report on "Some factors influencing root development" (1) by Hatton, Grubb and Amos, describes:

(a) the effect of several stocks and scions on the percentage of fibrous root of two year old apple trees;

(b) the effect of branch pruning on root development of one and two year old trees; and

(c) the effect of selective root pruning before planting.

Both stock and scion had some effect on the percentage of fibrous root. Branch pruning resulted in lessened root development. Removing roots before planting had less effect than branch pruning, but trees with all roots removed ("Stringfellowed") made less growth than those with roots left on.

Knight (4) in 1934 reported a more extensive trial of the effect of pruning young trees, in which similar results were obtained. He also showed that the ratio of new stem to new root remained remarkably constant whatever the pruning treatment, and further pointed out that in a large root system the percentage of fibre is naturally lower than in a small root system.

The study of the root systems of some mature fruit trees was commenced at East Malling Research Station in 1927 by the excavation of six ten year old Lane's Prince Albert apple trees. Three of these were on the vigorous rootstock Malling No. I (Broadleaved English) and three on the very dwarfing Malling No. IX (Jaune de Metz). A progress report describing the methods used and the results obtained was published in 1928 (6). In that report the hope was expressed that it might be found possible "Besides examining other rootstocks on the same soil . . . to investigate the development of similar stocks in other soils."

This has now been carried out with a number of rootstocks and on loam, sand and clay soils, with the results which are given in this paper.

II. MATERIAL, LOCALITY AND METHOD.

All of the twenty-six excavated trees described in this paper were of one variety—Lane's Prince Albert apple, a cooking variety of moderate vigour. The trees, which were all raised at East Malling Research Station, were on a wide range of rootstocks, from the very dwarfing No. IX (Jaune de Metz) to the very vigorous No. XVI and Long Ashton O.F.5 (unnamed). They were growing in the widely differing soils of Malling, Kent (loam), Wisley, Surrey (light sand), and Wisboro' Green, Sussex (heavy clay). All three series were growing in special stock trial plots, and their whole life history was recorded. They thus form a unique and truly comparable series.

Eleven trees were excavated from the Malling loam; four on stock No. I (Broadleaved English, classified as vigorous), two on stock No. II (Doucin, classified as semi-dwarfing), three on stock No. IX (Jaune de Metz, very dwarfing), and two on stock No. XVI (very vigorous). This work covered two winters, 1927-28 and 1928-29, when the trees were ten and eleven years old. The 1927-28 excavations consisted of two trees on No. I and one on

No. IX by the "skeleton" method, and one tree on No. I and two on No. IX by the "block" method. The remaining five trees were excavated by the block method in 1928-29. These methods are described below.

On the Wisley sand two trees on each of the main types, Nos. I. II, IX and O.F.5 (very vigorous), were excavated by the "combined" method in Winter 1928-29, and also one tree each on stocks Nos. IV, V, VII, X and XI. These trees were ten years old.

The heavy clay at Wisboro' Green limited the excavations on this soil to one tree on No. I and one on No. II in the Spring of 1930.

The soils are described in detail in the different sections that follow.

METHOD OF EXCAVATION.

The laborious nature of this work can be realized from the fact that over 1,000 tons of earth had to be finely broken and moved to excavate these twenty-six trees. In some cases more than sixty tons of soil had to be moved to uncover the roots of a single tree.

A trench was dug beyond the root area (found by trial) and it was then driven in sections of 50 cm. (about 20 inches)* across the ground occupied by the roots. The soil was gently worked away from the side of the trench with a small hand fork, leaving the roots exposed to view.

In the "skeleton" method the roots were kept intact as far as possible for reconstruction of the root system afterwards. In the "block" method, the soil was removed in blocks of known size and position as shown in Fig. 1, and the roots were removed from each block and sent to the laboratory to be washed, graded and weighed. In the "combined" method, the roots were cut and weighed in definite blocks, but were not subdivided into grades, so that reconstruction and photography of the root system was still possible.

The full technique is described in Appendix A.

III. DISCUSSION OF RESULTS.

The comparisons available from this work are between the root systems of Lane's Prince Albert apple on:

(A) The four main types of rootstock (described above) growing on loam soil at East Malling;

(B) Four main types of stock on sand at Wisley (and five other stocks); and

(C) Two of the main types on heavy clay at Wisboro' Green.

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* 100 cm. = 3·28 ft.

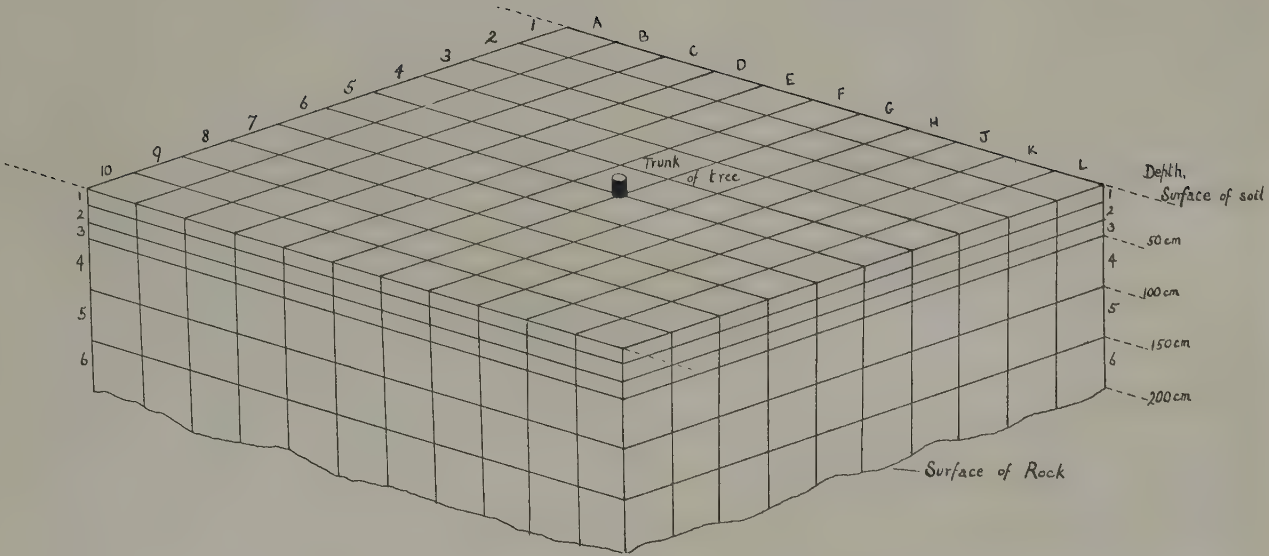

A
B
C
D
E
F
G
H
J
K
L
1
2
3
4
5
6
7
8
9
10
Trunk of tree
Depth.
Surface of soil
50 cm
100 cm
150 cm
200 cm
1
2
3
4
5
6
Surface of Rock

FIG. I.

Diagram showing arrangement of Blocks in Block method of excavation.

For the sake of simplicity, this illustration is given as an isometric projection, instead of as a perspective drawing.

There is also the comparison between the general type of root growth on the three soils and between the behaviour of individual stocks under these different soil conditions.

Validity of results.

It must be remembered that the results obtained are based on the excavation of only twenty-six trees in all, so that the number of replicates is necessarily small. The very costly and laborious nature of this work is, of course, responsible for this.

The qualitative observations are mainly quite evident when the root system is unearthed, and there is little doubt that they would apply generally under similar conditions. In the quantitative results, the good degree of agreement between the replicates encourages us to think that they too may form a good basis on which to work until a fuller series of excavations can be carried out.

As the work at East Malling was the most detailed, this will be considered first and the other localities compared with it.

A. THE FOUR MAIN TYPES OF ROOT STOCK ON LOAM SOIL AT EAST MALLING.

1. *Soil Profile.*

The soil profile of the East Malling Loam is described by Mr. G. H. Jones, in the Kent Fruit Soil Survey, as follows:

> "About 20 to 30 cm. of dark loam top soil on subsoil lighter in colour, of compact sandy loam to a depth of about 120 cm. or more. Below this occurred a very marked B horizon, which consisted of a layer of dark chocolate coloured greasy sandy clay, containing much glauconite and numerous minute air channels. This layer was of varying thickness, usually about 15 cm. and lay upon the eroded surface of the Ragstone rock of the Hythe Beds geological formation. The Ragstone is a calcareous sandstone rock, blue grey to yellow in colour. It occurs in alternate layers with uncemented material locally called Hassock, and forms a very free draining base to the soil. The dark layer, called Pug, is usually very moist, however. The depth of the Ragstone varied from about 100 cm. to 300 cm. below the trees excavated. The Ragstone underlies a large area of the fruit in Kent. The stone is used extensively for road making and building. The excavation area was practically level."

2. *General Characteristics of root and branch growth.*

Plates I and II are photographs of trees excavated from the Malling loam, and give a good idea of the amount and distribution of root and top on Stocks Nos. I and IX under these conditions.

In these photographs the large amount and spread of the vigorous No. I (Broadleaved English) root system, and the strong upright growth of the branches on this rootstock can be compared with the smaller amount of root of the No. IX, and the weaker more spreading type of branch growth it produces.

The way in which the larger main roots grew nearly horizontally, or sloped gently downwards or upwards in about the top 50 cm. of soil can be seen, and also the manner in which other roots of varying sizes strike vertically downwards from this scaffolding of main roots. At Malling these vertical roots grew right down to the Ragstone rock.

The spread of the roots is also seen to advantage in Figs. 2 and 3 which are root plans of trees on I and IX. The branch spread is marked by a dotted line, and it can be seen clearly how much farther the roots spread than the branches. It can also be seen how the roots by no means always grow in a straight line.

A rough comparison between the size of the trees on all four main types of rootstock on loam is given in Table I. While the gradation of size is clear, the comparison is not exact since the trees on II and XVI were a year older than those on IX and I, when excavated. Also the extreme root spread was not obtained from the trees on II and XVI, so the figure for ratio of branch spread to root spread is smaller than was actually the case. The figures will serve for contrasting with the size of tree on sand and clay given below, however.

TABLE I.

Rootstock Effect on Branch and Root Extent.

Lane's Prince Albert Apple on Loam Soil.

Rootstock No.	IX (very dwarfing).	I (vigorous).	II (semi-dwarfing).	XVI (very vigorous).
Age.	10 years.	10 years.	11 years.	11 years.
Branch height m.	2·1	2·9	2·7	3·5
Branch spread m.	3·2	3·5	3·7	4·6
Root spread m.	4·6	6·0	*5·0+	*6·0+
Root spread / Branch spread	1·43	1·71	*1·35+	*1·30+

* These trees were excavated by the Block method and the extreme root spread was not obtained.

Spreads are taken along and across the row. Figures are the means of two trees excavated.

Very detailed information as to the position of the roots is available from the trees excavated by the Block method, but it would be impossible to give

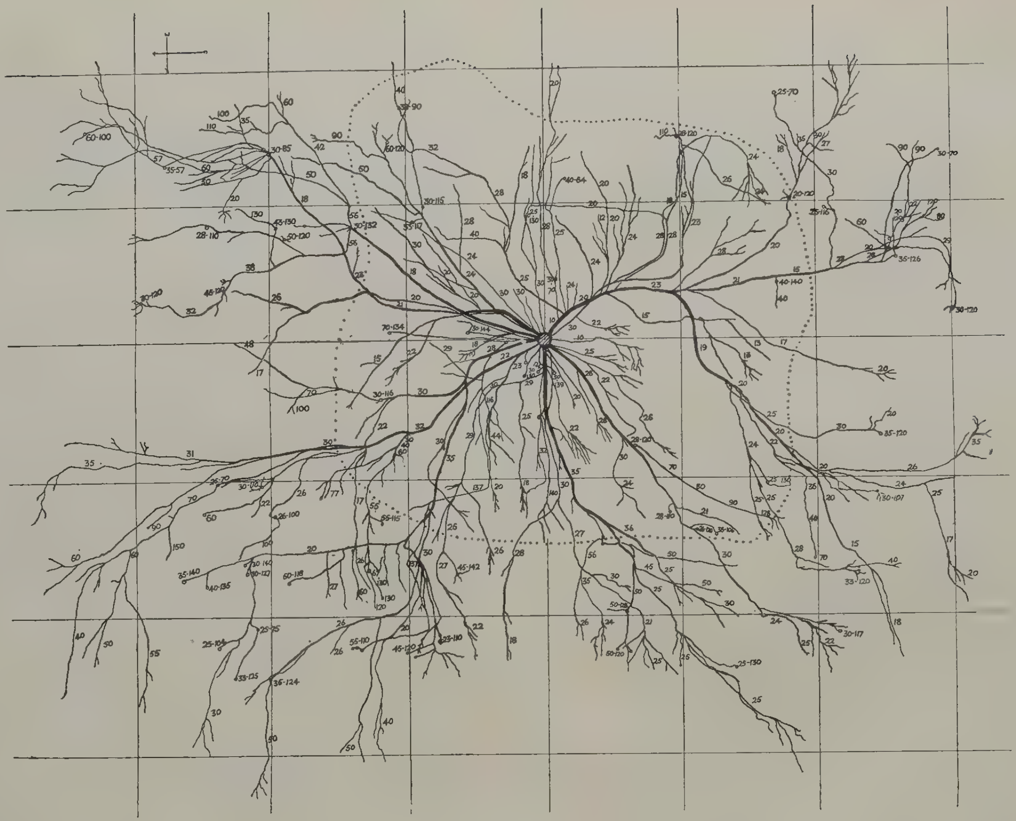


FIG. 2.

Root plan of ten year old Lane's Prince Albert on Stock No. I on Loam soil at East Malling. The plan is ruled in 1 metre squares. The numbers represent depth in centimetres of root below or to left. The dotted line indicates spread of branches. The circles indicate vertical roots, and the figures beside them the upper and lower limits of the root.

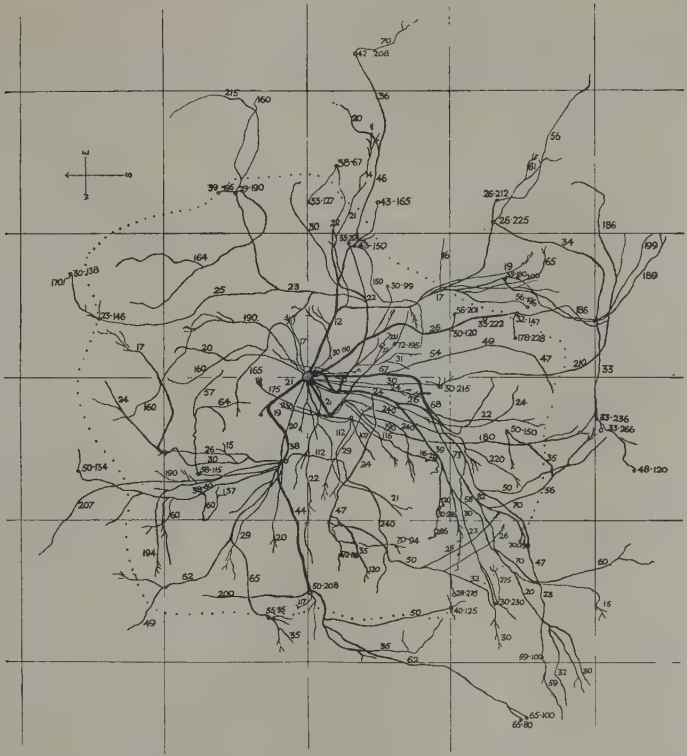

70
42 208
36
215
160
20
56
38·67
46
39
190
53·127
43·165
26·212
26·225
186
30
164
150
30·99
199
189
170
30·138
25
23
23·146
190
12
56·201
33·222
178·228
186
17
20
169
165
21
175
54
47
33
24
160
57
64
30·215
68
24
33·236
33·266
48·120
50·150
180
220
50·134
190
58·115
137
207
160
194
62
29
20
44
47
240
70·94
50
60
200
50·208
55·35
50
40·125
30·230
15
49
35
35
62
30
59·100
32
30
59
65·100
65·80

Fig. 3.

Root plan of ten year old Lane's Prince Albert on Stock No. IX on Loam soil at East Malling. The plan is ruled in 1 metre squares. The numbers represent depth in centimetres of the root below or to the left. The dotted line indicates spread of branches. The circles indicate vertical roots.

in full here the weights of each grade of root found in each of the 600 odd blocks of soil removed in the excavation of each tree. All that can be done is to summarize the data in various ways to show the main points of interest.

3. *Amount of Root.*

The total weight of root, and the amount in each grade for two trees of each of the main types of stock is shown in Table II.

The weight of root in a larger number of grades is available for most of the trees, and full details of seven grades for the three ten year trees were given in the progress report (6), but for brevity these are omitted here.

The relative vigour of the root systems can be seen from the total weights.

The very vigorous XVI's, for instance, have about five times the weight of root of the very dwarfing IX's, though they did not occupy nearly five times the volume of soil.

TABLE II.

Amount of Root produced by Lane's Prince Albert Apple on various Rootstocks on Loam at East Malling.

Rootstock.	Tree No.	Year excavated.	Age. Years.	Weight of roots gm.*			Total.
				Up to 1 mm. diameter.†	1 to 4 mm.	4 mm. and greater.	
No. IX	7·1	1927–28	10	628 *13·3%*	1,180 *24·9%*	2,931 *61·8%*	4,739
Jaune de Metz (Very dwarfing) ..	7·3	1927–28	10	808 *13·3%*	1,991 *32·7%*	3,294 *54·0%*	6,093
No. I	1·3	1927–28	10	1,963 *14·0%*	3,150 *22·4%*	8,917 *64·6%*	14,030
Broadleaved English (Vigorous) ..	1·5	1928–29	11	1,255 *6·7%*	3,088 *16·6%*	14,260 *76·7%*	18,603
No. II	1·6	1928–29	11	959 *6·6%*	2,452 *16·8%*	11,150 *76·6%*	14,561
Doucin (Semi-dwarfing)	1·7	1928–29	11	1,101 *7·0%*	2,410 *15·3%*	12,277 *77·7%*	15,788
No. XVI	14·2	1928–29	11	1,923 *6·4%*	4,240 *14·2%*	23,773 *79·4%*	29,936
Un-named (Very vigorous) ..	15·2	1928–29	11	1,714 *6·8%*	3,864 *15·3%*	19,691 *77·9%*	25,269

* 1,000 gm. = 2·2 lb. † called "fibre."

4. *Relative amount of roots of the different grades.*

It will be observed that the percentage of "fibre" varies not so much between different rootstocks as between the different years of excavation. It is unlikely that this was due to the different graders used in the two years, since the result of the "calibration" described in Appendix A indicated that the 1927-28 grader and recorder would have given values for fibre some 9 per cent. less than the 1928-29 graders and recorders, whereas, in point of fact, the value for fibre in 1927-28 was about 100 per cent. greater, i.e. 13·3 to 14·0 per cent. as against 6·6 to 7·0 per cent.

It is possible, therefore, that there was an actual large difference between the amount of fibre in the two seasons. Kolesnikov has shown (5) that a great deal of root may die off during the Winter and one of the writers has found (10) that the root system of a young apple stock may decrease considerably in weight during one Winter and but little another Winter. When mortality occurs amongst roots it would be the finer roots—especially the "fibre" that might be expected to suffer most.

It will not be safe, however, to conclude that such large variations in percentage of fibre occur normally from season to season, until fuller experimental evidence on the point is forthcoming. There seemed to be no significant differences between the various stocks with respect to the relative amounts of root of various grades. Such differences as occur between the amounts in the higher grades are fully accounted for by differences in the total size of the trees, for a large root system may obviously be expected to have a higher proportion of roots of the greater diameters.

5. *Radial Distribution of Roots.*

In the progress report published on the results of the 1927-28 excavation, a " horizontal projection " of the root system of each tree was presented. This consisted of a horizontal plan of the excavated area. The plan was divided up into squares, each square representing a vertical prism of soil 50 cm. square, running down from the surface of the soil to the surface of the rock. In each square was entered the value for the total weight of roots found in that prism and the corresponding value for the weight of Grade I roots or fibre.

Similar horizontal projections have been prepared for all the trees excavated at East Malling, Wisley and Wisboro' Green, except that the values for fibre are only available for the Malling trees.

On account of expense, however, these plans cannot all be published here, but diagrams for eleven year old trees on Malling No. II, No. I and No. XVI are given in Appendix B.

The diagrams show that there is not great dissimilarity between the different stocks in their radial distribution of root, except that the high amount of fibre usually found round the collar of No. I stock is seen—the central square metre holding 173 gm. fibre as compared with 61 gm. on the No. II and 84 gm. on the No. XVI.

Fig. 4 summarizes the percentages of root weight in the various soil regions for two trees on each stock. These areas are the same as those bounded by heavy lines in Appendix B, viz., the column of soil one metre square surrounding the trunk, the hollow column three metres square, that five metres square, and that outside five metres square.

It can be seen that the total root weight falls off very rapidly from the trunk outwards, while the fibre is much more evenly distributed over the whole root area. Whereas 33 to 43 per cent. of the total root weight was found in the central square metre, this region only held about 10 per cent. of the fibre. Except in the case of the dwarf trees on No. IX the trees had some 50 to 60 per cent. of their fibre outside the three metre square surrounding the trunk.

6. *The Feeding Roots.*

The large roots must be considered as the anchors of the tree and as the main scaffolding and food conducting tubes of the root system. The actual

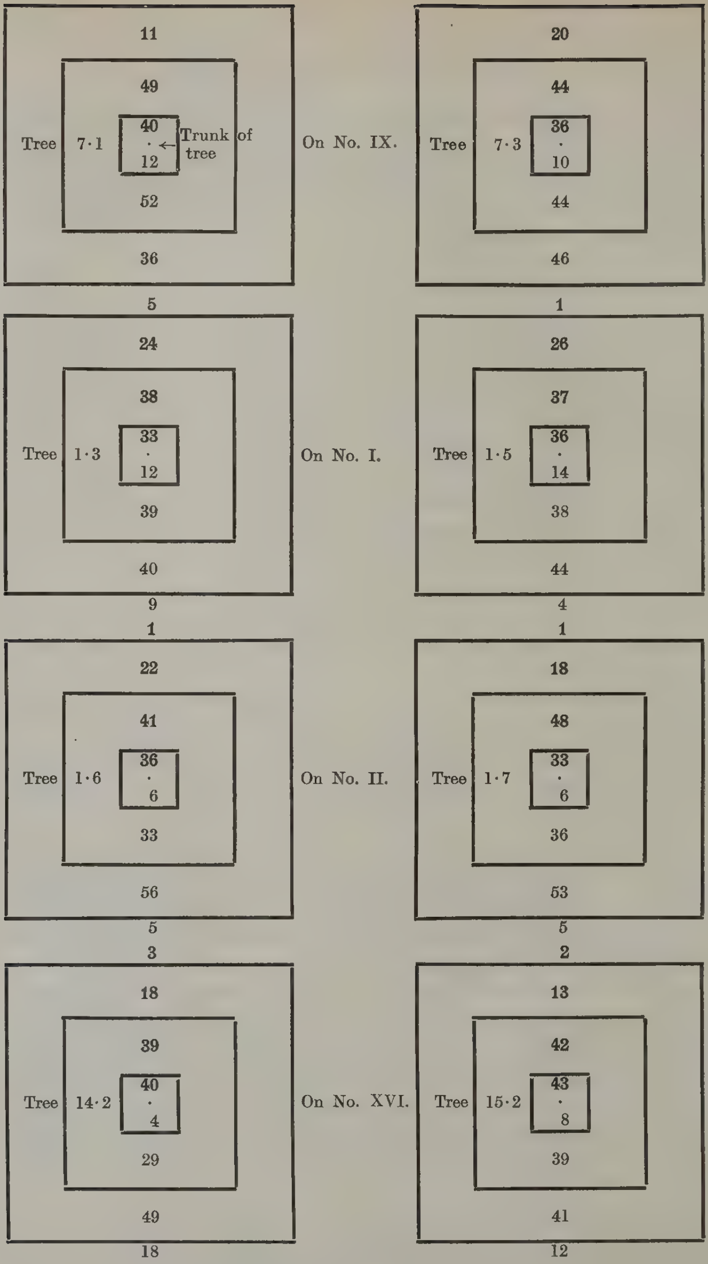

11
49
Tree
7·1
40
Trunk of tree
12
52
36
5
On No. IX.
20
44
Tree
7·3
36
10
44
46
1
24
38
Tree
1·3
33
12
39
40
9
On No. I.
26
37
Tree
1·5
36
14
38
44
4
1
22
41
Tree
1·6
36
6
33
56
5
On No. II.
1
18
48
Tree
1·7
33
6
36
53
5
3
18
39
Tree
14·2
40
4
29
49
18
On No. XVI.
2
13
42
Tree
15·2
43
8
39
41
12

FIG. 4.

Radial distribution of root on Loam soil.

Percentages of total root weight and of fibre in different regions of root system. Upper values (in Clarendon type, thus **11**) are percentages of total root weight; lower values are percentages of fibre. In each figure the central square represents the column of soil 1 metre square surrounding the trunk of the tree. The next square the hollow column of soil 3 metres square immediately surrounding the central column. The outer square a hollow column of soil 5 metres square.

absorbing tips appear to be borne mainly on the smaller fibrous roots. Even the weight of fibre is but a crude measure of the distribution of absorbing surface of the roots, but is probably the best measure at present available. The wide distribution of the fibre emphasizes the necessity for placing fertilizers over the whole root area, extending even well past the branches, if most efficient feeding is to be obtained.

These excavations have shown that, under Malling conditions with trees planted at 15 ft. square, in eleven years there is hardly a cubic foot of soil that is not occupied by some roots in the case of all stocks excavated, except the very dwarfing No. IX.

7. *The One-sidedness of the Root Systems.*

Both the maps which showed the course of the main roots and the plans which showed the distribution of root weights over the excavated area, demonstrated the very striking asymmetry frequently shown by the root systems.

This is brought out clearly by Fig. 5, in which the root system of each tree at East Malling has been divided up into its four quadrants by means of lines running N—S and E—W, intersecting at the centre of the trunk. In each quadrant has been entered the percentage of the total root weight that occurred in it, the corresponding values for fibre being entered in light type.

Under these conditions, the roots show no consistent bias in any one particular direction that could be attributed say to sunshine, shade or prevailing wind. Their direction seems to have been a combination of chance and the various soil factors. It is clear that a root pruning operation which consisted of removing roots in any one quadrant of the soil might cut off a very variable proportion of the total root system.

8. *Depth of Roots.*

The remarkable depth of all the root systems at East Malling has already been commented on, and is visible in Plates I and II. In all cases the vertical roots grew right down to the Ragstone rock, or the sandy layer of uncemented rock material (called Hassock) which occurred in a few places above it. They then often turned horizontally and ramified profusely in the layer of clayey soil just above the rock or Hassock. This clayey layer is an excellent example of a "B Horizon" or accumulation layer. The roots of one of the trees on No. IX reached a maximum depth of 296 cm. (over 9′ 6″) in a depression of the rock which happened to occur below this tree. Below the other trees the rock depth varied from about 100 cm. to 200 cm. and thus limited the root depth. There is evidently neither lack of air nor excess of water in this soil, right down to the rock, and the limit to root depth is in most cases the mechanical one of the hard rock surface. Where the Hassock occurred, the roots did not

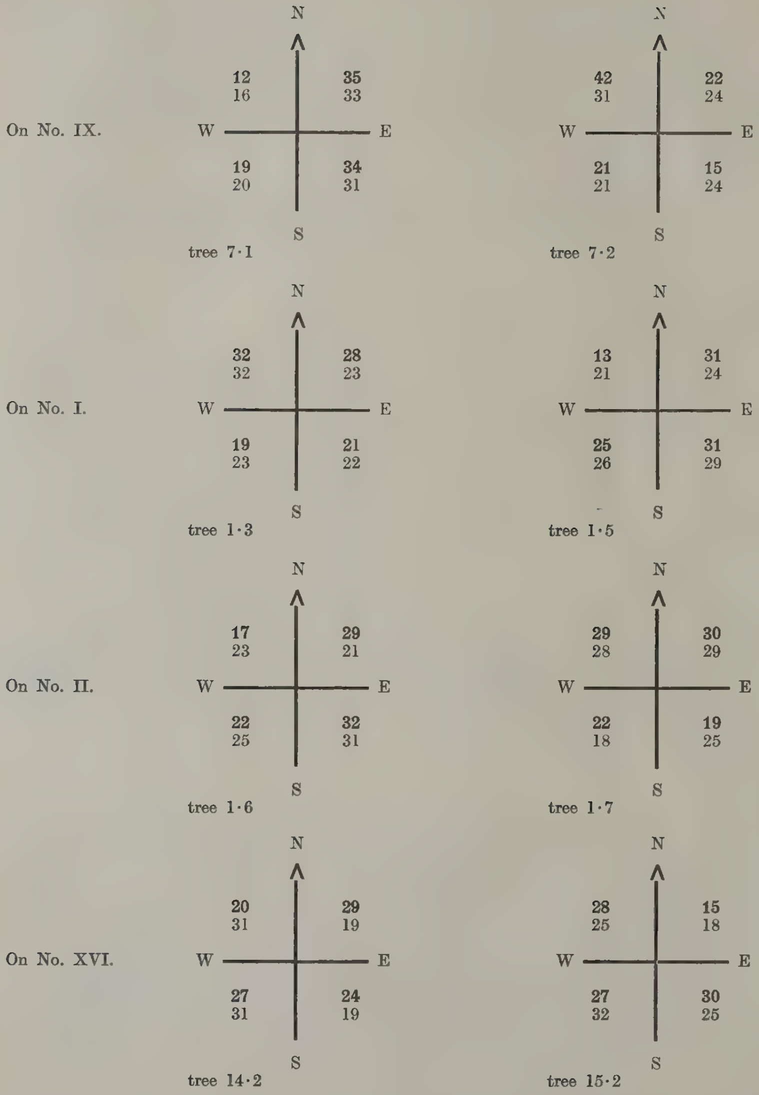

On No. IX.
N
12
16
35
33
W
E
19
20
34
31
S
tree 7·1
N
42
31
22
24
W
E
21
21
15
24
S
tree 7·2
On No. I.
N
32
32
28
23
W
E
19
23
21
22
S
tree 1·3
N
13
21
31
24
W
E
25
26
31
29
S
tree 1·5
On No. II.
N
17
23
29
21
W
E
22
25
32
31
S
tree 1·6
N
29
28
30
29
W
E
22
18
19
25
S
tree 1·7
On No. XVI.
N
20
31
29
19
W
E
27
31
24
19
S
tree 14·2
N
28
25
15
18
W
E
27
32
30
25
S
tree 15·2

Fig. 5.

One sidedness of root systems on Loam soil. Percentages of root weight in the four quadrants of each root system. Upper values (Clarendon type) = percentage of total root weight. Lower values = percentage of fibre.

grow in it to any great extent although it is loose and sandy, and this was probably due to the larger amount of moisture and nutrient in the soil layer just above. Some stocks planted in pots of the raw Hassock grew well for three years, showing that it is definitely not toxic to the roots.

TABLE III.

Percentage of Root Weight in different depths on Loam Soil (Malling).

Stock No.	IX.		II.		I.		XVI.	
Tree No.	7·1	7·3	1·6	1·7	1·3	1·5	14·2	15·2
Total root weight gm. Fibre weight gm.	**4,739** 628	**6,093** 808	**14,561** 959	**15,788** 1,101	**14,031** 1,963	**18,603** 1,255	**29,936** 1,923	**25,269** 1,714
Depth	Percentage of root weight.							
0–17 cm.	**9** 10	**7** 18	**7** 14	**8** 12	**12** 21	**14** 26	**31** 26	**13** 21
17–34 cm.	**40** 14	**40** 15	**47** 15	**39** 18	**61** 33	**58** 23	**47** 19	**59** 18
34–50 cm.	**11** 9	**20** 11	**13** 10	**19** 9	**8** 9	**5** 12	**7** 14	**7** 11
50–100 cm.	**25** 36	**15** 23	**25** 44	**21** 39	**12** 21	**12** 26	**13** 35	**16** 36
100–150 cm. ..	**12** 24	**13** 22	**8** 17	**13** 22	**7** 16	**11** 13	**2** 6	**4** 12
150 cm. to rock ..	**3** 7	**5** 11	**—** —	**—** —	**—** —	**—** —	**—** —	**1** 2
Total percentage below 34 cm. ..	**51** 76	**53** 67	**46** 71	**53** 70	**27** 46	**28** 51	**22** 55	**28** 61
Total percentage below 50 cm. ..	**40** 67	**33** 56	**33** 61	**34** 61	**19** 37	**23** 39	**15** 41	**21** 50

The ***heavy type*** refers to Total Root.

The ***light type*** refers to Fibre, i.e. root below 1 mm. diameter.

The percentage of root weight of each tree in the various layers of soil is shown in Table III. The figures in heavy type refer to total root weight, and those in light type refer to the fibre.

It will be noticed that the two trees of each stock resemble each other in relative depth fairly closely. There is also a certain resemblance between the trees on Stocks No. IX and No. II, and between the trees on No. I and No. XVI. This is brought out clearly in the totals at the bottom of the table, where it is seen that Nos. IX and II had 46 to 53 per cent. of their total root weight and 67 to 76 per cent. of their fibre more than 34 cm. deep, while Nos. I and XVI had only 22 to 28 per cent. of their total root weight and 46 to 61 per cent. of their fibre more than 34 cm. deep. This is of interest since

Nos. IX and II are in the Dwarfing and Semi-dwarfing groups of stocks, and Nos. I and XVI are classed as Vigorous and Very Vigorous respectively. The fibre is much more evenly distributed in depth than the total root, as was the case in radial spread. The figures for distribution of total root weight are largely controlled by the bigger main roots, which occurred near the trunk. In the case of the No. XVI, these were very large and heavy, while in the case of No. I stocks, they were also quite large and numerous. It is notable that No. I has a larger percentage of its fibre in the top layers of soil than any of the other stocks. This is probably because of the profuse mat of fibre which is found round the collar and the main roots of this stock. Even in the case of the No. I, 37 to 39 per cent. of the fibre was more than 50 cm. deep, while the No. IX and No. II stocks had 56 to 68 per cent. of their fibre more than 50 cm. deep, and the No. XVI stocks 41 and 50 per cent. of their fibre beneath this depth. It is therefore quite easy to understand that fertilizers applied to the surface of the soil may take several years to show their full effect.

Unfortunately, the unevenness in the depth of soil undoubtedly affects the depth distribution figures of the roots to a considerable extent, and makes it impossible to draw the conclusion that the dwarfing stocks are habitually deeper rooted than the vigorous ones. It is clear, however, that the dwarfing of the scion is not necessarily due to a shallow root system—as an old tradition held to be the case—for the trees on No. IX showed all the characteristics of dwarf trees, although their root systems were the deepest found.

Similarly, the trees on No. XVI were typical very vigorous trees, although their roots could not get to a very great depth on account of the relative shallowness of the rock below them.

9. *Relation of Root to Tree Performance.*

The weights of roots, branches and fruits of each tree are summarized in Table IV, and the relation between them is shown in the lower half of the table.

The vigour of trees as shown by branch weight covered the large range from 12,200 gm. on No. IX to 76,204 gm. on No. XVI. The ratio of stem weight (i.e. trunk and branches) to root weight (columns g and h) however, is remarkably constant. In nearly every case the stems (at the time of lifting) weighed $2\frac{1}{4}$ to $2\frac{1}{2}$ times as much as the roots. The trees on No. I had a slightly lower stem/root ratio, but with such small numbers of trees this difference can hardly be taken as significant. In fact, in this case, the difference between the stocks does not lie in the relative amount of stem growth per unit of root weight, but in the total amount of growth of both root and stem. In this the rootstock has evidently been the controlling factor, since the scion variety is the same in each case. The fruit production should also be considered in this connection however. Column k shows that the trees on No. IX

TABLE IV.

Root, Branch and Fruit Data. *Lane's Prince Albert Apple on Loam Soil.*

	Rootstock	IX.	IX.	I.	I.	II.	II.	XVI.	XVI.
	Tree No.	7·1	7·3	1·3	1·5	1·6	1·7	15·2	14·2
	Date when lifted	Jan. 1928	Jan. 1928	Jan. 1928	Nov. 1928	Nov. 1928	Dec. 1928	Jan. 1929	Dec. 1928
	Age when lifted	10 years	10 years	10 years	11 years	11 years	11 years	11 years	11 years
a	Weight of branches and trunk when lifted	12,200	14,050	27,100	39,009	33,566	34,836	59,375	76,204
b	Weight of prunings to date	1,701	2,480	4,590	6,653	4,795	5,277	9,560	10,398
c	Total stem growth weight	13,901	16,530	31,690	45,662	38,361	40,113	68,935	86,602
d	Total root weight when lifted	4,739	6,093	14,031	18,603	14,561	15,788	25,269	29,936
e	Total stem and root	18,640	22,623	45,721	64,265	52,922	55,901	94,204	116,538
f	Total weight of fruit	58,600	71,900	77,600	114,728	95,522	90,983	119,154	78,840
g	$\frac{c}{d}$ $\frac{\text{Total stem}}{\text{Root}}$	2·93	2·71	2·26	2·45	2·63	2·54	2·73	2·89
h	$\frac{a}{d}$ $\frac{\text{Stems (as lifted)}}{\text{Root}}$	2·57	2·31	1·93	2·10	2·31	2·21	2·35	2·55
j	$\frac{f}{e}$ $\frac{\text{Fruit}}{\text{Total tree weight}}$	3·14	3·18	1·70	1·79	1·80	1·63	1·26	·68
k	$\frac{f}{d}$ $\frac{\text{Fruit}}{\text{Roots}}$	12·4	11·8	5·53	6·17	6·56	5·76	4·72	2·63
l	$\frac{f}{c}$ $\frac{\text{Fruit}}{\text{Total stem}}$	4·22	4·35	2·45	2·51	2·49	2·27	1·73	·91

stocks produced about 12 Kg. of fruit per Kg. of root, while the trees on No. XVI only produced about 3·5 Kg. of fruit per Kg. of root—both stocks having at the same time produced about 2·8 Kg. of stem per Kg. of root. This difference probably lies mainly in the precocity of the dwarfing stocks. The trees on No IX, as is characteristic of this stock, began to fruit many years before the trees on No. XVI. The urge to early fruit production certainly comes from the No. IX stock, so though the "efficiency" of No. IX is not greater than that of the other stocks in making more branch per unit root weight, up to this age its "relative productiveness" is certainly greater in that it induces a greater proportion of the tree's early efforts to be turned to fruit production rather than wood production.

One interesting point appears; since these early-cropping trees with a high fruit/tree-weight ratio and the late-cropping trees with a low fruit/tree-weight ratio both have a similar stem/root ratio, it is reasonable to infer that fruit is produced by the use of material that would otherwise be used for the formation of both new stem and new root, and not at the expense of either stem alone or root alone. This point cannot be proved finally until other trees of various ages and at different stages of maturity have been examined, but it is very suggestive.

B. THE FOUR MAIN TYPES OF ROOTSTOCK ON LIGHT SAND (WISLEY, SURREY).

1. *Soil Profile.*

This plot was chosen because it was one of the sandiest patches of the Bagshot Sands formation. The profile was usually about 30 cm. of darkish brown light sand, sometimes extending to 50 cm. with yellowish sand below to about 150 cm. becoming very compact towards the base. Wavy strata were visible, but they appeared to be different only in colour. Below the subsoil there was a darker band of hard sand, almost sandstone, about 10 cm. thick and then there was soft white sand leading to a hard clayey layer with pebbles at the base at about 240 cm. Excavation of roots in the top metre was quite easy, the sand wall being so loose that it would fall if walked upon, but below 1 metre every inch of sand had to be dug away with a fork or pick.

The excavation area had a slight slope to the N.W.

2. *General Characteristics of Trees.*

The general appearance of the roots and branches of the four main types of rootstock on this very poor sandy soil are shown in Plates III to VI, which are scale photographs. It will be seen at once that the trees on sand are much smaller than the comparable trees on loam (Plates I and II). In fact the two less vigorous stocks Nos. IX and II have been unable to make a good sized tree at all. The more vigorous I and O.F.5 have made a small and medium size bush tree respectively in ten years. The relative vigour of the stocks has not been changed by the poor soil, but the whole series has been made much smaller, as shown by the measurements in Table V.

TABLE V.

Rootstock Effect on Branch and Root Extent.

Lane's Prince Albert Apple, ten years old, on poor Sandy Soil.

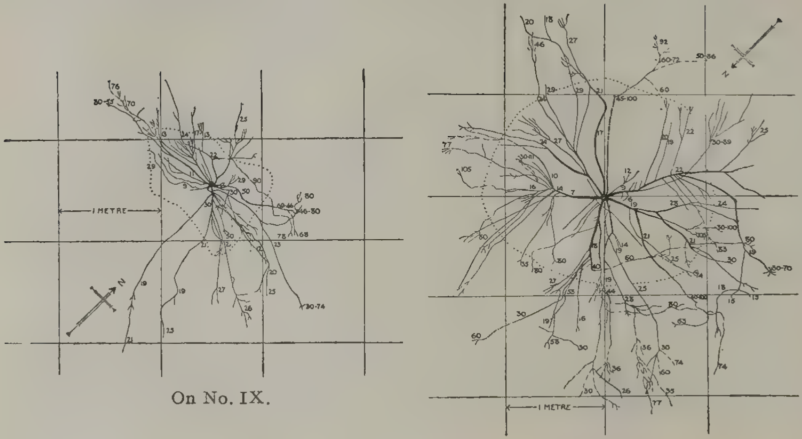
Rootstock No.	IX.	II.	I.	O.F.5.
Branch height cm. ..	105	114	149	250
Branch spread cm. ..	124	142	190	328
Root spread cm.	235	313	438	920
Ratio $\frac{\text{Root spread}}{\text{Branch spread}}$ ..	1·9	2·2	2·3	2·8

The figures give the mean of two trees. Branch and root spread were taken in two directions at right angles.

3. *Root Spread.*

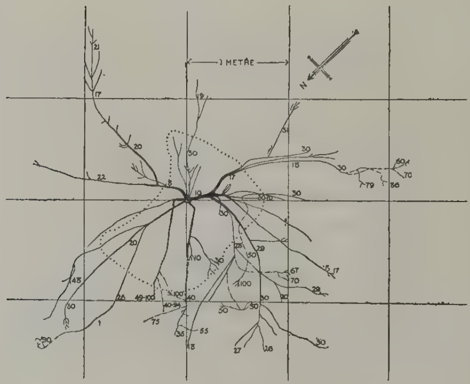
The most striking feature of the root systems is their great spread. The mean of two diameters at right angles is shown in Table V, and it will be seen that the root spread is 1·9 to 2·8 times the branch spread. The best comparison, however, is in the root plans, Figs. 6 and 7, where it can be seen what a very large area the roots had to draw upon in order to support a relatively small head (shown by the dotted lines). The largest trees had a mean root spread of 9·2 metres (over 30 ft.). As the trees were planted at 3 metres apart, there was considerable root interlacing, especially in the case of the O.F.5 (Fig. 7). The large amount of root interlacing made the use of the "block" method of excavation quite impossible on this soil. The interlacing roots are omitted from the plans, except in the case of Fig. 7, where one root from an adjacent tree A (North-West side) is shown, as it grew in a rather peculiar way. This root from tree A grew straight up to the trunk of tree E and became firmly attached to it, but grew on for about two feet beyond. Thus the two trees were actually united by one root. In the case of tree D (South-West side), a root from tree E grew up to and beyond its trunk, but had not become attached. It had merely become bent in a bow shape as the trunk expanded.

The distance from tree E to the next tree on the North-East side (B) was 4·5 metres instead of 3 (on account of a pathway). This is reflected in a greater root growth in that direction, showing that root competition was a definite factor in affecting root spread.



On No. IX.

On No. I.



On No. II.

FIG. 6.

Root plans of ten year old Lane's Prince Albert apple on rootstocks IX, I and II. Compare with Fig. 7. The dotted line indicates branch spread. The figures indicate depth of root. The small circles show vertical roots, and the figures beside them show the upper and lower limit of the root.

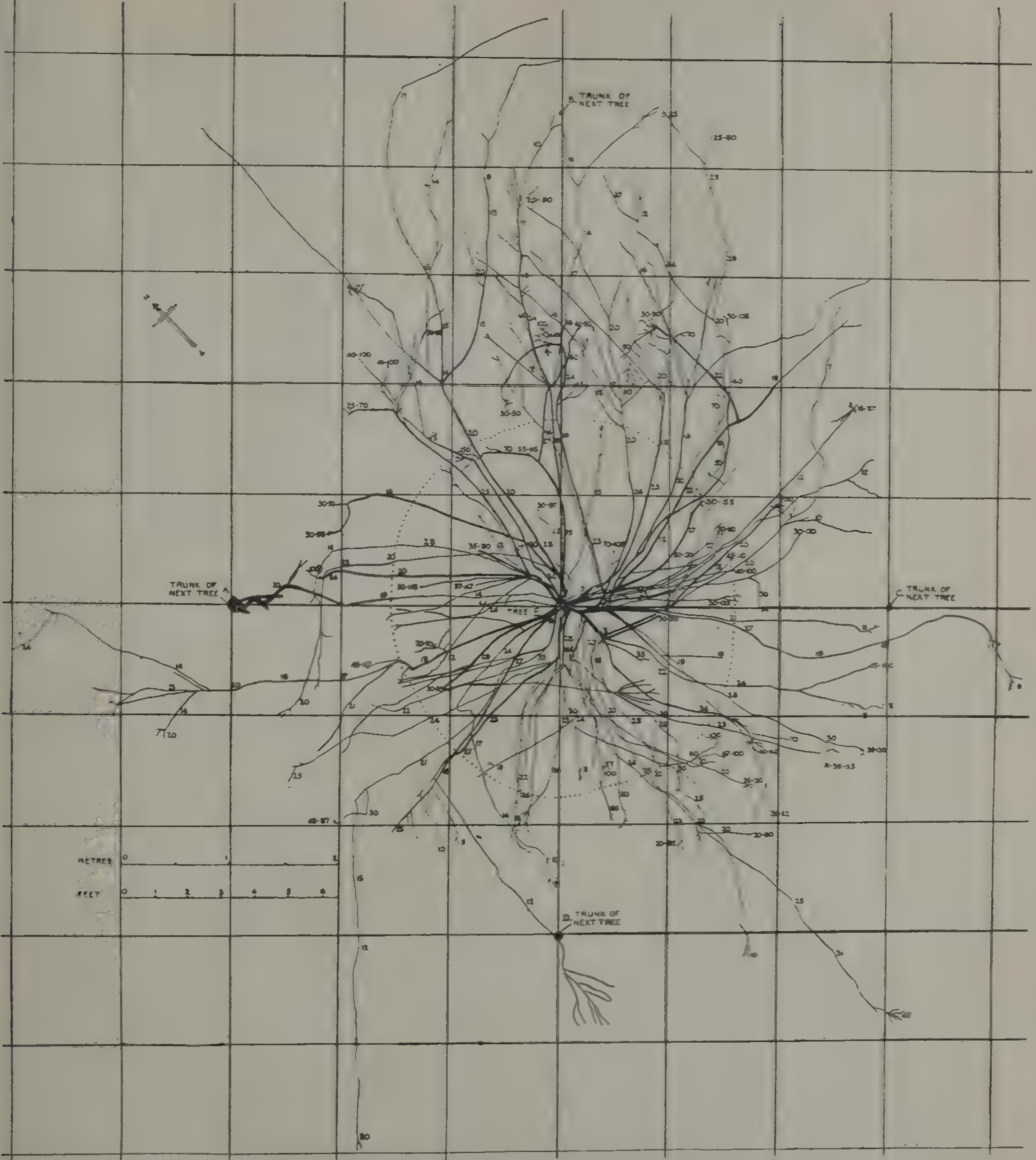

TRUNK OF NEXT TREE B
TRUNK OF NEXT TREE A
TRUNK OF NEXT TREE C
TRUNK OF NEXT TREE D
TREE
METRES 0 1 2
FEET 0 1 2 3 4 5 6

Fig. 7.

Root plan of ten year old Lane's Prince Albert apple on rootstock O.F.5. Compare with Fig. 6. The dotted line indicates branch spread. The small circles show vertical roots, and the figures beside them show the upper and lower limit of the root.

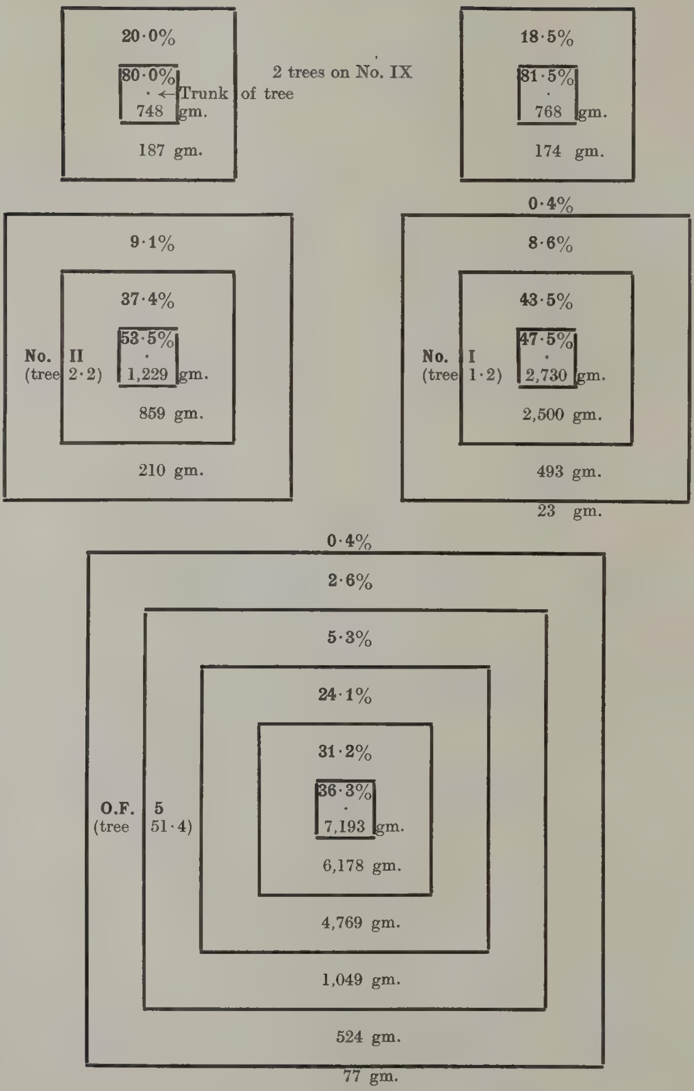

20·0%
80·0%
← Trunk of tree
748 gm.
187 gm.
2 trees on No. IX
18·5%
81·5%
768 gm.
174 gm.
9·1%
37·4%
53·5%
No. II
(tree 2·2)
1,229 gm.
859 gm.
210 gm.
0·4%
8·6%
43·5%
47·5%
No. I
(tree 1·2)
2,730 gm.
2,500 gm.
493 gm.
23 gm.
0·4%
2·6%
5·3%
24·1%
31·2%
36·3%
O.F. 5
(tree 51·4)
7,193 gm.
6,178 gm.
4,769 gm.
1,049 gm.
524 gm.
77 gm.

FIG. 8.

Radial distribution of root on Light Sandy soil.

Lower figures refer to total root weight, upper figures to percentage of total root weight in the regions shown, which are squares of side 1 m. and hollow squares of side 3 m., 5 m. etc. surrounding the trunk.

The radial distribution of root is shown quantitatively in Fig. 8. The great difference in size of tree given by the different rootstocks naturally causes a much higher percentage of total root to appear in the centre square metre around the weaker stocks than around the stronger stocks. This tendency is much more marked on the sand than upon the loam (Fig. 4) owing to the greater contrast in size of tree.

4. *Root Depth.*

It can be seen from the photographs (Plates III to VI) that the root systems on the sand were not nearly so deep as those on the loam. The same general conformation is visible, however. There was a shallow scaffolding of main roots, usually less than 30 cm. deep, and a number of roots descending more or less vertically, which penetrated to a maximum of 80 cm. in the case of the No. IX, 137 cm. in the case of the No. II, 115 cm. in the case of the No. I, and 190 cm. in the case of the No. O.F.5 stocks. These figures are the extreme depths, and many of the vertical roots only reached to about half these depths.

Here there was no hard rock to stop the roots, and the sand continued to a depth of at least 6 metres, with the various strata already noted.

The limit to root depth was therefore fairly certainly not mainly a mechanical one, but is probably connected with the physiological factors of nutrients and moisture.

The trees on the very vigorous O.F.5 stock apparently had the initial vigour to penetrate to the moist clayey layer, and access to this moisture supply doubtless helped them greatly in times of drought, when the top soil became practically loose blowing sand.

Some of the deeper roots at Wisley ended in a curious bunch of swollen fibrous roots, which often ended sharply at a certain level. These were quite distinct from the ordinary white growing tips, and their cause has not yet been determined. It is possible that the seasonal rise of a water table may have some connection with the abrupt ending and bunching of these roots.

Some further information on root depth is given in Table VI (b) which shows the percentage of root weight above and below 30 cm. Only two depth divisions were made in these excavations, and the fibre was not detached since the reconstruction of the root systems was the main consideration, and, for the same reason, the roots in the centre block were left attached to the trunk, and their weight was calculated.* Too much stress cannot therefore be laid on

* The volume of the base of the trunk to which the roots were attached (the top having already been cut off and weighed) was estimated by careful measurements. This volume was then multiplied by a factor found, by experiment, to be the approximate sp.g. of the wood, and the resulting weight was deducted from the total weight of trunk with roots attached. In some examples in which the roots were detached entirely this method of calculation was found to give a result very close to the actual weight found by weighing.

these weight figures. It is striking, nevertheless, to note that even the deepest tree in this series had only 27 per cent. of its root weight below 30 cm., and the shallowest 6·2 per cent. (The trees on Nos. IV and VII were rather deeper, however, as shown below.)

TABLE VI.

Rootstock Effect on (a) Root and Stem Weight
(b) Root Depth
of Lane's Prince Albert Apple, ten years old, on poor Sandy Soil.

Stock No.	IX.		II.		I.		O.F.5.	
Tree No.	9·4	9·5	2·1	2·2	1·1	1·2	51·4	51·5
(a) Root and Stem weight.								
Stem weight gm. (trunk and branches)	917	843	1,360	1,751	2,069	5,309	14,326	14,330
Root weight gm. ..	939	942	1,907	2,298	2,717	5,746	19,790	19,771
Ratio $\frac{\text{Stem weight}}{\text{Root weight}}$	0·98	0·89	0·71	0·76	0·76	0·92	0·72	0·72
(b) Root depth.								
Percentage of root weight :								
above 30 cm. ..	81·6	93·8	—	79·6	—	75·4	79·7	73·0
below 30 cm. ..	18·4	6·2	—	20·4	—	24·6	20·3	27·0

5. *Relation of root to tree weight.*

Table VI (a) shows the root and stem weight for each stock, and the relation- between them. The great contrast between the stocks is clearly seen. The greatest variation within a stock is shown by the two trees on No. I, which were rather above and below the average respectively. The two trees on O.F.5 are amazingly alike in root and stem weight.

As the total tree weight varied from about 1·8 Kg. on the No. IX stocks to 34·1 Kg. on the O.F.5, it is interesting to note that the stem/root weight ratio only varied from 0·71 to 0·98.

This compares with a stem/root ratio of 1·93 to 2·58 at Malling. It can be seen, therefore, that on the poor sandy soil at Wisley nearly three times as much root was required to support a given amount of branch as was required on the loam at Malling.

The fruit weights for these trees are not available for the whole period, so no fruit/root ratios are given. The relative precocity of cropping of the stocks was the same as at Malling, however.

The data from the trees on stocks Nos. IV, V, VII, X and XI have not been included in full for the sake of simplicity. They agreed in general with the trees of the four main types given. No. V (Doucin Amelioré) was of about the same size as the No. II, and was similar in depth. The Nos. X and XI were rather similar to No. I. There was much fine root round the trunk of No. X, but the large main roots seemed rather bare of fibre. The No. XI on the other hand had much fine rootlet along its main roots, giving them quite a "furry" appearance compared with other stocks. Nos. IV and VII both had a branch weight of about 8,000 gm., i.e. a little over half that of the O.F.5. The tree on No. IV had a very one-sided root system. It is interesting to note, however, that although No. IV is notorious for its poor anchorage, this tree had 29·8 per cent. of its root weight below 30 cm. (i.e. slightly more than the vigorous well anchored O.F.5 stocks). Its deepest roots reached 118 cm. below ground level. It is quite possible, therefore, that a poor anchorage is not always due to a shallow root system.

The No. VII was remarkable for the large amount of comparatively deep root. Many of its roots were 50 to 100 cm. deep, and 41·9 per cent. of its root weight was more than 30 cm. deep. It would be dangerous to conclude from this single example that No. VII is habitually much deeper rooted than other stocks, however.

C. TWO ROOTSTOCKS ON HEAVY CLAY SOIL (WISBORO' GREEN, SUSSEX).

1. *Soil Profile.*

The soil here is upon the Sussex Weald and forms a complete contrast to the Wisley sand.

The top soil was about 30 cm. of dark clay passing into a wettish plastic mixture of red and white material to about 90 cm., here reddish mottling increased and the soil became distinctly drier, having bands of brown flaky material, quite hard but almost crumbly. At 150 cm. it was so dry that it fell off the auger (in April).

The ground sloped gently to the East, and was reported to have been pipe drained many years ago. No drains were found in the excavation trench, but water appeared at about 90 cm. in places.

2. *General Characteristics of Trees.*

Plate VII shows an eleven year old Lane's Prince Albert apple tree on Stock No. II, excavated from the heavy clay soil. The characteristic root formation on this soil is seen to be very different from that on loam or sand. The root system is much more restricted, the roots taper and divide up very rapidly, and the depth distribution is characteristic, as discussed below. The size of tree is a little smaller than on the loam but larger than on the sand, as shown in Table VII.

TABLE VII.

Rootstock Effect on Branch and Root Extent.

Lane's Prince Albert Apple, eleven years old, on heavy Clay Soil.

Rootstock No.	II.	I.
Branch height cm.	265	285
Branch spread cm.	276	308
Root spread cm.	440	510
Ratio $\frac{\text{Root spread}}{\text{Branch spread}}$	1·59	1·66

3. *Root Spread.*

Table VII shows that the roots spread about 1·6 times as far as the branches on the clay soil. The actual position of the roots can be seen in the plans (Fig. 9), which also show the relative size of the two trees.

The diagrams in Fig. 10(*a*) show that 48 per cent. to 49 per cent. of the total root weight was in the metre square surrounding the trunk, which compares with 33 to 36 per cent. on the loam. Beyond the 3 metre square the No. I has 10 per cent. of its root weight, compared with 25 per cent. on the loam, and the No. II has 7 per cent. compared with 20 per cent. This shows how much more relatively compact the root systems were on the clay, for the branch spread was not much smaller on the clay than on the loam.

4. *One-sidedness.*

The one-sidedness of root systems is shown very clearly in Fig. 10(*b*), which gives the percentage of root weight in the various quadrants. The No. I has in the four quadrants 51 per cent., 12 per cent., 6 per cent. and 31 per cent. of its root weight, i.e. more than half the root system occurred in a single quadrant.

5. *Depth of Rooting.*

The most noticeable feature of the root systems on the clay was the very clearly marked depth boundary. The clay extended to a very great depth, but the root system appeared to be sharply cut off at 90 to 100 cm., though one or two fine roots, probably of the current season's growth, grew beyond this to a maximum of 118 cm. deep. Several fairly large roots, which had grown vertically downwards, had evidently been killed back from the tip upwards to about 80 or 90 cm. below the surface (Plate VIII). These roots had continued to thicken, and had sent out new rootlets from the tip in the following year, only to have them killed off again to about the same depth. Other main roots had curved round into a horizontal position between 50 and 90 cm. deep, as seen in Plate VIII.

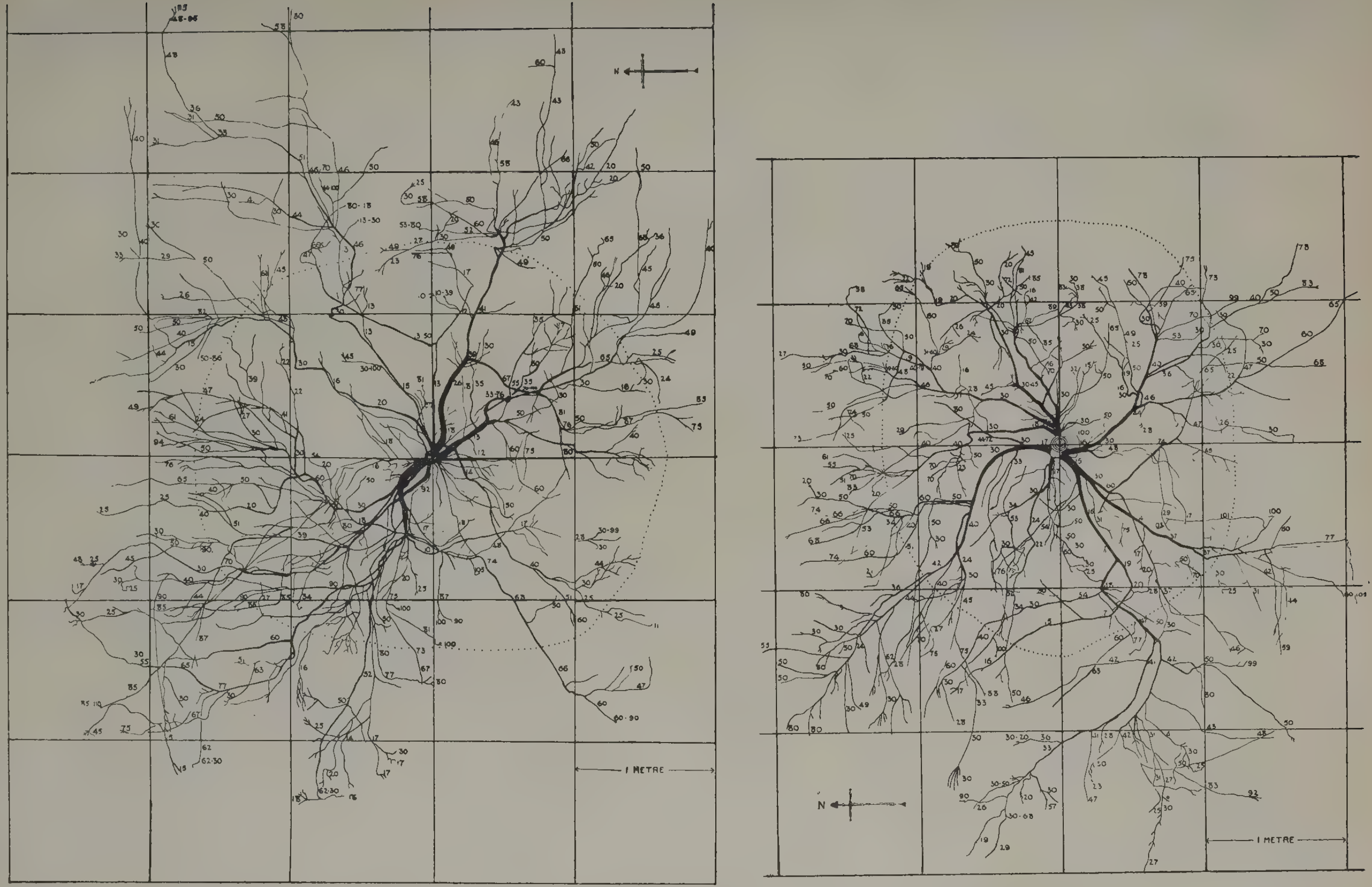
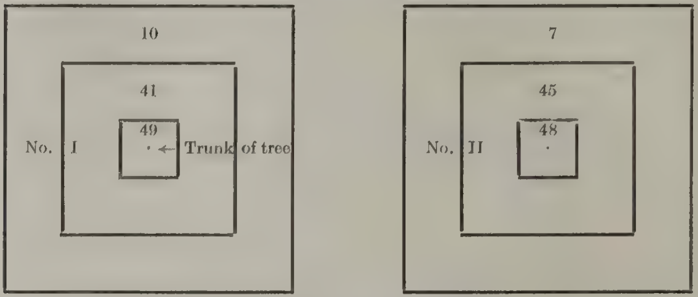


FIG. 9.

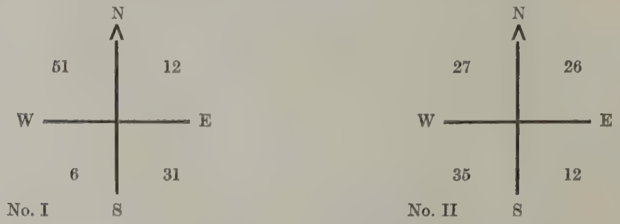
Root plans of eleven year old Lane's Prince Albert apple on Clay soil (Wisboro' Green).
(Left) On Stock No. I. (Right) On Stock No. II.
The figures indicate root depth.

Fig. 10.

Radial distribution of roots, and one-sidedness of root systems on Clay soil.



(*a*) Percentages of total root weight in the soil regions shown (*cf.* Fig. 4).



(*b*) Percentages of total root weight in the four quadrants of the root system.

The main scaffolding was definitely deeper than on the sand or the loam. Fine bunches of root grew upwards into the top soil, but the main roots evidently preferred the sticky subsoil to the top soil or the soil below 100 cm. Most of the main roots were between 30 and 60 cm. deep. The reason for this is not very clear. The top soil was cultivated to a depth of about 12 cm., and a layer of old leaves turned in by ploughing was clearly noticeable at this depth. It may be that the roots avoided the top soil either because of excessive wetness in Winter or because of excessive dryness in Summer. Large cracks become very prominent on this soil in the summer months. The lower boundary suggests a winter water table. During the excavations (March 1930) free water was noted to ooze out at depths between 70 and 90 cm. in several places. One curious point was that below 100 cm. the soil was drier at the time of the excavation. In fact, at 150 and 180 cm. it was so dry that it fell off the auger. Whatever the actual limiting factor may have been, it is clear from the position of the roots that the layer of soil between about 30 and 90 cm. was more congenial to them than the soil above or below. This also agrees with the root distribution found in another clayland excavation (7).

The actual percentage of root in the various layers is seen in Table VIII. The high percentage between 30 and 50 cm. deep is noticeable, viz., 24·6 per cent. for the No. I and 40·2 per cent. for the No. II, compared with 5 to 8 per cent. between 33 and 50 cm. deep on No. I on loam and 13 to 19 per cent. for No. II on loam.

TABLE VIII.

Depth of Rooting of ten year old Lane's Prince Albert Apple on heavy Clay Soil.

Rootstock	No. II.	No. I.
Percentage in depth .. 0–30 cm.	50·2	53·7
30–50 cm.	40·2	24·6
50–100 cm.	9·6	21·6
Below 100 cm.	—	0·1
Total root weight gm. ..	7,177	8,592

The bark of the roots in the clay was very dark—almost black in colour. On the sand and loam it was brown. In some places on the clay the soil just round the roots was also blackened, and had a curious "sour" smell, similar to that observed in another clay excavation (7). This smell and also the blackening of the soil gradually passed off on exposure to light and air.

TABLE IX.

Root, Branch and Fruit Weights of ten year old Lane's Prince Albert Apple on heavy Clay Soil.

Stock	II.	I.
Stem weight gm. .. .. (trunk and branches)	15,150	18,700
Root weight gm.	7,177	8,573
Total Fruit weight gm. ..	52,800	61,000
Ratio $\frac{\text{Stem weight}}{\text{Root weight}}$	2·11	2·18
Ratio $\frac{\text{Fruit weight}}{\text{Root weight}}$	7·36	7·12

6. *Root, Branch and Fruit Weight.*

The weight of branches, roots and total fruit of the two trees are shown in Table IX. The tree on No. I is heavier than that on No. II, as before. Both trees are less than half the weight of the comparable trees on the Malling loam (a year older).

The ratio of stem weight to root weight is 2·11 for the II and 2·18 for the I. This is about the same as on the loam, and more than twice that on the sand.

The fruit is a little more than half that borne at Malling, and the fruit/tree-weight ratio and the fruit/root-weight ratio are slightly greater than at Malling.

IV. CONCLUSIONS.

The great range of vigour and precocity obtainable by the use of different rootstocks has already been shown in several reports. The effect of different soils in modifying the growth of the whole range of stocks while not altering their relative vigour has also been discussed. It has remained for this excavation work to show how the different root systems develop in the soil, and what their relation is to the tree performance.

The mature root systems of the different rootstocks have shown distinctive characters in their type of root, in most cases similar to the characters observable in the young plants. No. I, for instance, has a large mass of fibrous root round the collar and main roots (*cf.* Plates I and IV); No. II tends to have fewer main roots, which are usually coarse and fangy (*cf.* Plate III); No. IX has a much thicker root cortex than the other rootstocks. We were actually able to distinguish the No. IX roots from interlacing roots of other trees by the noise the No. IX roots make when broken between the fingers. They give a sharp snap on account of their thick cortex, while other roots do not break nearly so sharply. O.F.5 has very numerous and wiry roots. While all the root systems were modified to some extent by the different soils, their general characteristics remained so distinctive that there was little possibility of mistaking them.

This shows that we are still dealing with different varieties of root, even though they have borne the same scion variety for years, and that each type of root has its own inherent possibilities which it will use to the full under the modifying influence of environmental factors.

This work has shown, however, that the ratio of stem weight (i.e. trunk and branches) to root weight in mature trees has remained remarkably constant throughout the range of rootstocks examined on a given soil. Thus a little tree on a dwarfing stock will have a light root system, and a large massive tree on a vigorous stock a correspondingly massive root system. Up to the age of ten years the more dwarfing rootstocks have produced a greater weight of fruit per unit of root weight than the more vigorous rootstocks, however. It therefore appears that the more dwarfing stocks have shown greater "relative productiveness" up to this age in that they have turned a larger proportion of the tree's growth energy to fruit production rather than wood growth, while maintaining the same stem/root ratio as the more vigorous and tardy cropping stocks.

Knight (4) and Vyvyan (11) have shown that in young trees the new weight added during the growing season is distributed in fixed proportion between new stem and new root, and Knight found that this proportion was more or less the same within a variety on a number of rootstocks and with different pruning treatments. The finding of a constant stem/root ratio in mature trees in the present work therefore implies that this constant ratio of stem increment to root increment has been maintained as the tree gets older. This is a most important point, which throws new light not merely as Vyvyan (11) suggests on a possible explanation of rootstock influence on the scion, but also on the inevitable effect of branch treatments on the root and of root treatments on the stems throughout the life of the tree.

That the stem/root ratio should be different on different soils is also important, though perhaps only to be expected, since the root environment is completely changed in different soils, while the branch environment (the air) remains more nearly the same. Similarly, it has been shown in a previous report (8) that the stem/root ratio of pears on a given soil was modified by manuring.

The Root and the Soil.

Of the many soil factors that affect the root growth, we have been able to note a few. In the case of root depth at Malling, the mechanical limiting factor of hard rock has been shown. On the clay at Wisboro' Green the physical factor of a water table is suggested, causing root limitation by cutting off aeration. The long thin roots in the sand demonstrate growth unimpeded mechanically, while the short curving roots in the clay demonstrate growth in a stiff medium. The fact that in many cases two or more roots grew almost alongside suggests some particularly congenial combination of conditions in certain spots. The factor of root competition is also demonstrated, for although much interlacing of individual roots from adjoining trees was found, the root plans show that except when relatively small the root systems tend on the whole to occupy a square or rectangular space limited by the root systems of adjoining trees. The absence of root competition on one side is one factor accounting for the frequent abnormal performance of headland trees.

A further factor of great importance is the soil moisture. Monthly moisture determinations at five depths were made for a year in the excavation orchard at East Malling. Similar determinations were started at the other two centres, but had to be discontinued owing to distance. The figures are given in Table X. Unfortunately moisture equivalent figures are not available, so the texture of the soil must be allowed for in making comparisons. The records give a fair comparison of the increase or decrease of moisture at the various depths from month to month however.

In most cases three borings were made. A composite sample was made for layer 1, and separate determinations were made for each sample in the other layers. (The means are given in Table X.) There was a much greater variability in the lower layers than in the upper ones, and the standard errors are as follows: Layers 2 and 3, S.E. 0·34, Significant difference 0·96; layers 4 and 5, S.E. 0·98, Significant difference 2·76. A significant difference between adjacent values is marked with a colon thus (:). The figure of 0·96 obtained for layers 2 and 3 has also been applied to layer 1.

It will be noted that at Malling the sticky "pug" layer just above the rock (i.e. the layer from 117 to 133 cm. at the spot where the determinations were made) has contained more moisture than the upper layers throughout the year. This is doubtless one of the reasons for the deep roots ramifying profusely in this layer. It appears that the rainfall of 2·73 inches in the month preceding June 14th has almost made up for the transpiration of the tree since there was little decrease of soil moisture from 17 to 133 cm. deep; but the small rainfall of 0·68 inches in the next month ending July 11th was reflected in the considerable drying out of the soil at all depths. The very heavy rainfall in the next period practically replenished the reservoir, however, right down to the rock. After a small further drying out in September the winter rains once more gradually filled up the soil reservoir. The dry March winds began to dry out the top soil without greatly affecting the deeper layers.

The two months' determinations at Wisley and Wisboro' Green show the great contrast in water retaining capacity of these two soils. They also show that the greatest percentage of moisture at that time was in the 50-67 cm. layer in both cases, and that the deeper layers were definitely dryer.

Much fuller information is required before very definite correlations between soil moisture and root growth can be made. Some further knowledge on this point is being obtained in the Root Observation Trenches now installed at East Malling (9).

In the field it is evident that the factors affecting the root growth are numerous, and the final reaction of the root appears to be to grow anywhere it can in order to get what it wants.

Unfortunately space will not permit the inclusion of many points of minor interest—the weed roots, the worm holes, the new soil mites and various insects observed during the course of the work. Nor to tell of the time when the frost gradually hardened our excavation wall into a solid mass which could not be moved even with the aid of a burning coke bucket; nor the time when heavy rain over the week-end weighed down our tarpaulin shelter into a miniature swimming bath. But these and many other items all added to the zest of discovery given by the unearthing of these root systems, and helped to lighten a work which at times seemed to require an inexhaustible supply of patience and perseverance.

TABLE X.

Soil Moisture.

Percentage Moisture to Fresh Weight of Sample.

	Malling Loam.				1930.				1931.				*Wisley Sand.* 1930.		*Wisboro' Clay.* 1930.	
Depth.	May 9	June 14	July 11	Aug. 15	Sept. 11	Oct. 17	Nov. 18	Dec. 15	Jan. 15	Feb. 20	Mar. 18	Apr. 17	May 8	June 13	May 8	June 13
0–17 cm.	15·8 :	12·7 :	8·5 :	14·3	14·1 :	16·1	16·5	16·7	16·6	16·8 :	15·0	15·7	6·9	5·8	18·2 :	15·8
17–33 cm.	14·3	14·3 :	11·6 :	13·8	13·0 :	14·2	14·8	15·3	15·3	15·5 :	14·1	14·5	7·4	7·2	19·3 :	16·9
50–67 cm.	13·4	13·0 :	11·6 :	13·4 :	12·3 :	13·5 :	14·8	15·4 :	13·9	14·5	14·0	13·8	8·5	8·6	21·7 :	20·2
80–100 cm.	14·5	14·3 :	11·0 :	14·0	13·2	16·2	15·8	15·9	14·5	16·0	16·8	15·5	6·0	7·0	20·7	20·5
117–133 cm.	17·6	17·6	16·1	17·5	16·9	19·8	19·7	18·8	20·8	20·0	20·2	17·5	5·3	5·2	18·0	18·7
Rainfall in previous monthly period—inches.	1·42	2·73	0·68	4·93	1·19	3·34	2·61	4·45	1·53	2·53	0·69	1·14				
	Standard error. Layers 2 and 3, 0·34 Standard error. Layers 4 and 5, 0·98					Significant difference 0·96 Significant difference 2·76										

A colon, thus : indicates significant difference between adjacent values.

ACKNOWLEDGMENTS.

Grateful thanks are due to the authorities of the Royal Horticultural Society and to A. J. Carter, Esq., New Pound, Wisboro' Green, for permission to excavate the trees from the stock trial plots at Wisley and Wisboro' Green respectively, and for providing facilities for carrying out the work on these plots.

The writers are much indebted to several members of Staff for assistance with the recording and other work connected with this report. Special mention should also be made of Mr. W. Horton's careful and patient work in the actual unearthing of the roots.

V. SUMMARY.

1. The complete root systems of twenty-six Lane's Prince Albert apple trees, ten and eleven years old, on various clonal rootstocks, were excavated from three soils: Loam, Sand and Clay.

The root systems covered a very wide range of vigour, from very dwarf (e.g., Malling No. IX) to very vigorous (e.g., No. XVI). The trees were planted as stock trial plots, and form a truly comparable series.

The extent, amount and position of the root systems and their relation to tree performance and to the soil profile was studied. These points are illustrated by photographs of reconstructed root systems, and by root plans, besides diagrams and tables.

2. In all cases the roots spread farther than the branches. In the sand the root spread was two to three times the branch spread. In the loam and clay it was about one and a half times the branch spread. The ratio of root spread to branch spread was slightly higher in the more vigorous than in the more dwarfing stocks.

3. The fine fibrous root occurred everywhere and grew in all directions including upwards. It was distributed over the whole root system. Only about 10 per cent. of the fibre was in the square metre of soil surrounding the trunk of ten and eleven year old trees on loam. Nearly half the fibre was outside the 3 metre square surrounding the trunk. Hence, for most efficient feeding, manure needs to be spread over the whole root area, reaching beyond the branch spread.

4. The root depth varied considerably on the different soils. On the loam there was a fairly shallow scaffolding of main roots about 20 to 33 cm. deep, from which numerous vertical roots descended to the rock at all distances from the trunk. These roots often turned horizontally along the rock and ramified profusely in the sticky B horizon just above it. The rock occurred at depths varying from 100 to 300 cm. below these trees. The deepest roots found reached 296 cm. (over 9′ 6″). These were on Stock No. IX.

5. On Stocks IX (very dwarfing) and II (semi-dwarfing), about half the total root weight and three quarters of the fibre was below 33 cm. and three fifths of the fibre was below 50 cm. Stocks I (vigorous) and XVI (very vigorous) were relatively shallower, having only a quarter of their total root weight and half their fibre below 33 cm., and about two fifths of the fibre below 50 cm. This variation in depth was probably partly due to deeper rock below Stocks IX and II.

It is clear that a dwarf tree is not necessarily shallow rooted, for in this case the smallest trees had the deepest roots, and the largest trees had the shallowest. The great depth of many of the roots explains why manures applied to the surface may take several years to show an effect.

6. The roots on the sand were shallower than on the loam. The same general conformation of a shallow scaffolding with vertically descending roots was found. In this case there was no clearly defined depth boundary. The maximum depths found were No. IX, 80 cm.; No. II, 137 cm.; No. I, 115 cm.; No. O.F.5, 190 cm. In most cases more than three quarters of the total root weight was less than 30 cm. deep.

7. In the clay the main scaffolding of roots was deeper than on the sand or loam. Most of the roots sloped down and grew along in the sticky subsoil between 30 and 60 cm. deep. Branch roots grew upwards and downwards. There was a very sharply marked depth boundary at about 90 cm. Though a few young roots had penetrated to 118 cm., there were signs of killing back to the 90 cm. level. This suggests a seasonal water table cutting of aeration.

8. The trees varied greatly in weight and in the amount of fruit they had produced, according to the stock on which they were worked and the soil on which they grew. The ratio of stem weight (i.e. trunk and branches) to root weight was comparatively constant on a given soil, however, irrespective of the size of the tree and the crop it had borne. The stem/root weight ratio varied between different soils. On the loam it was from about 2·0 to 2·5, on the clay it was about 2·1, and on the sand it was from about 0·7 to 1·0. Hence it requires more than twice as much root to support a given amount of top on the poor sand as on the loam or clay.

9. The type of root was modified by the soil. In the sand the roots were long, thin, straight and spreading. In the clay the roots were fairly short, tapering, rapidly branching, and twisting in all directions. In the loam they were intermediate in form between those in sand and those in clay.

10. In spite of the modifying influence of the soil the roots of the different stocks were recognizable by their distinctive characteristics. Neither the soil nor the scion variety had eliminated the original stock differences in ten years.

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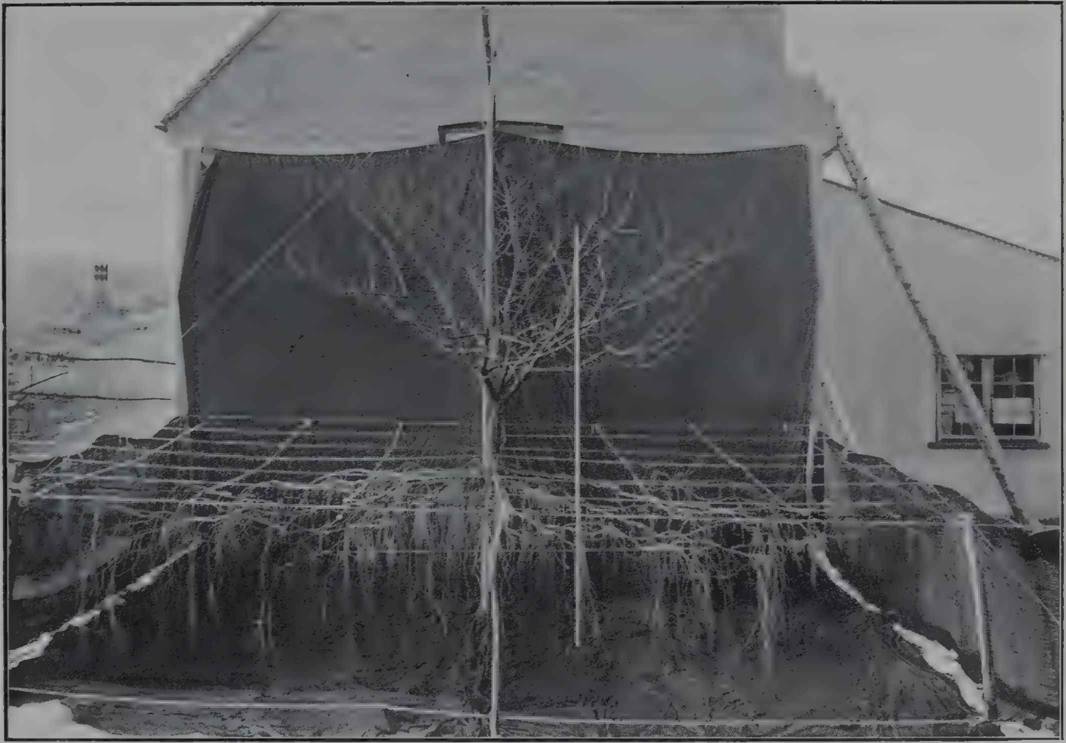
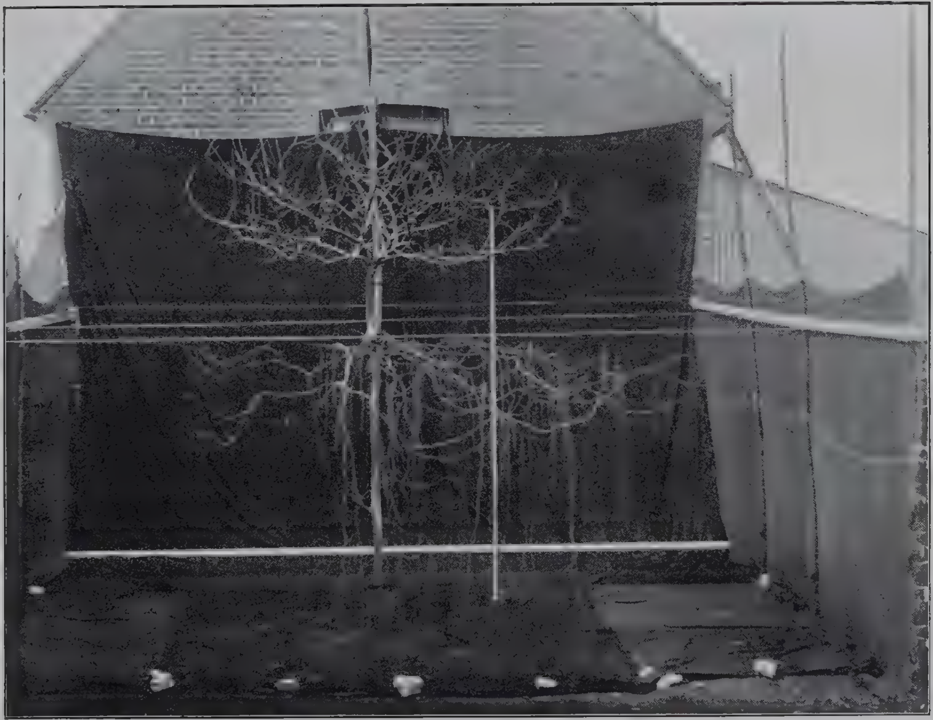
PLATE I.



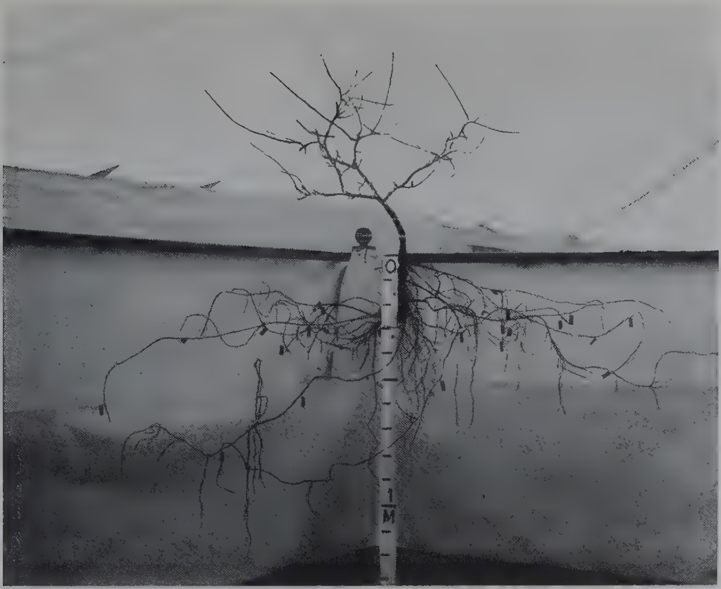
Ten year old Lane's Prince Albert apple on rootstock No. I excavated from Loam soil at East Malling.
The white strings form 1 metre squares at ground level.

PLATE II.



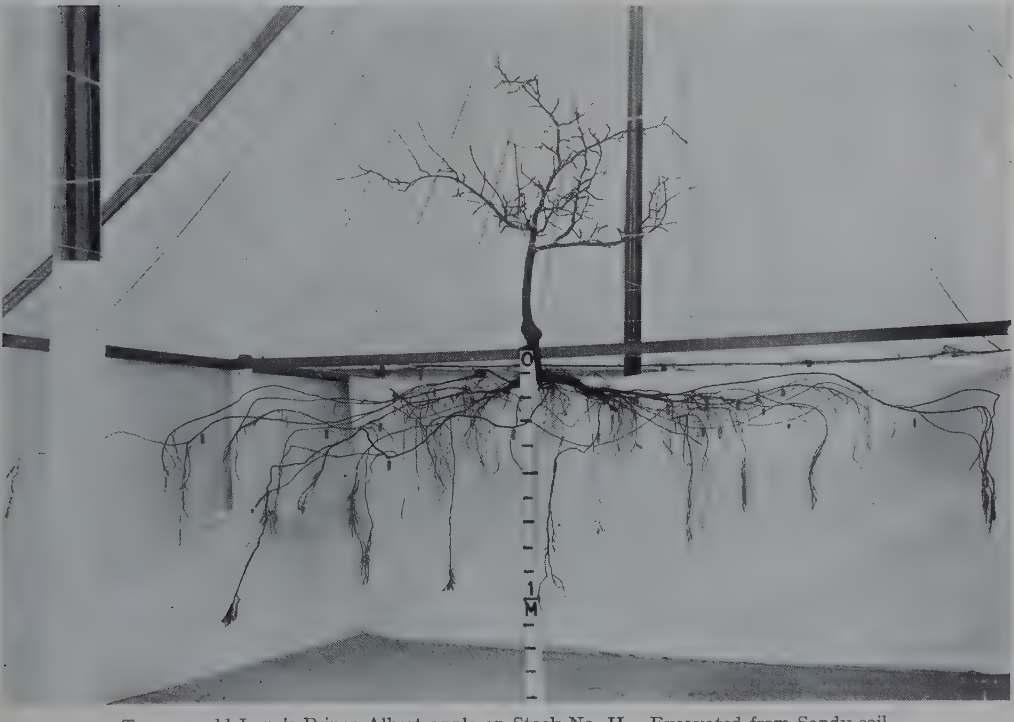
Ten year old Lane's Prince Albert apple on rootstock No. IX, excavated from Loam soil at East Malling. The white measure is 3·35 m. long.

PLATE III.



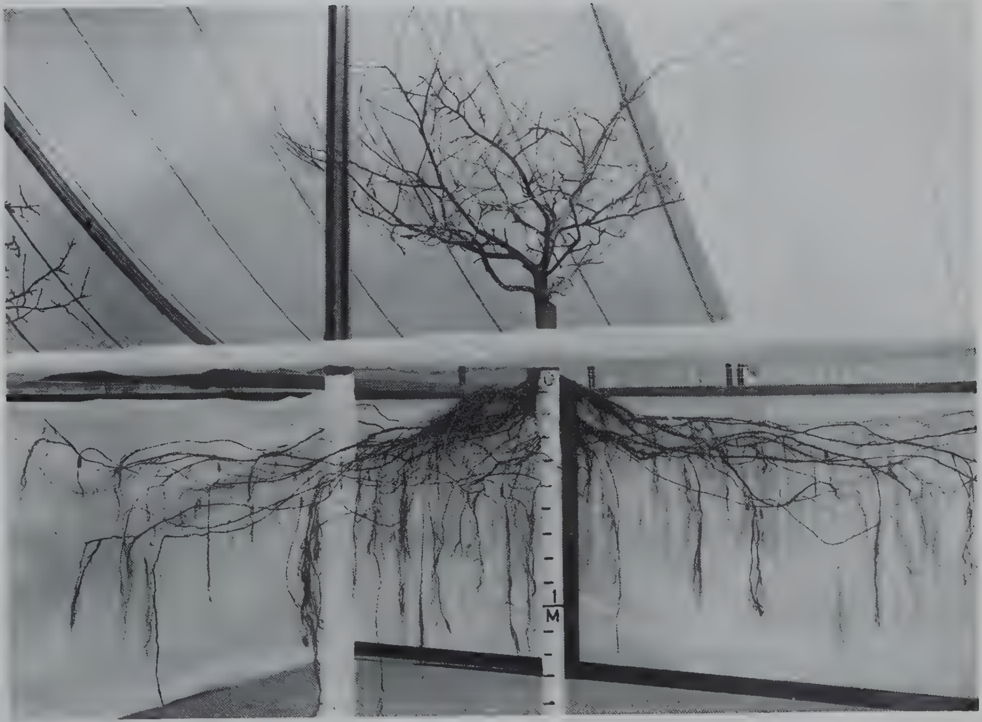
Ten year old Lane's Prince Albert apple on Stock No. IX. Excavated from Sandy soil.

PLATE IV.



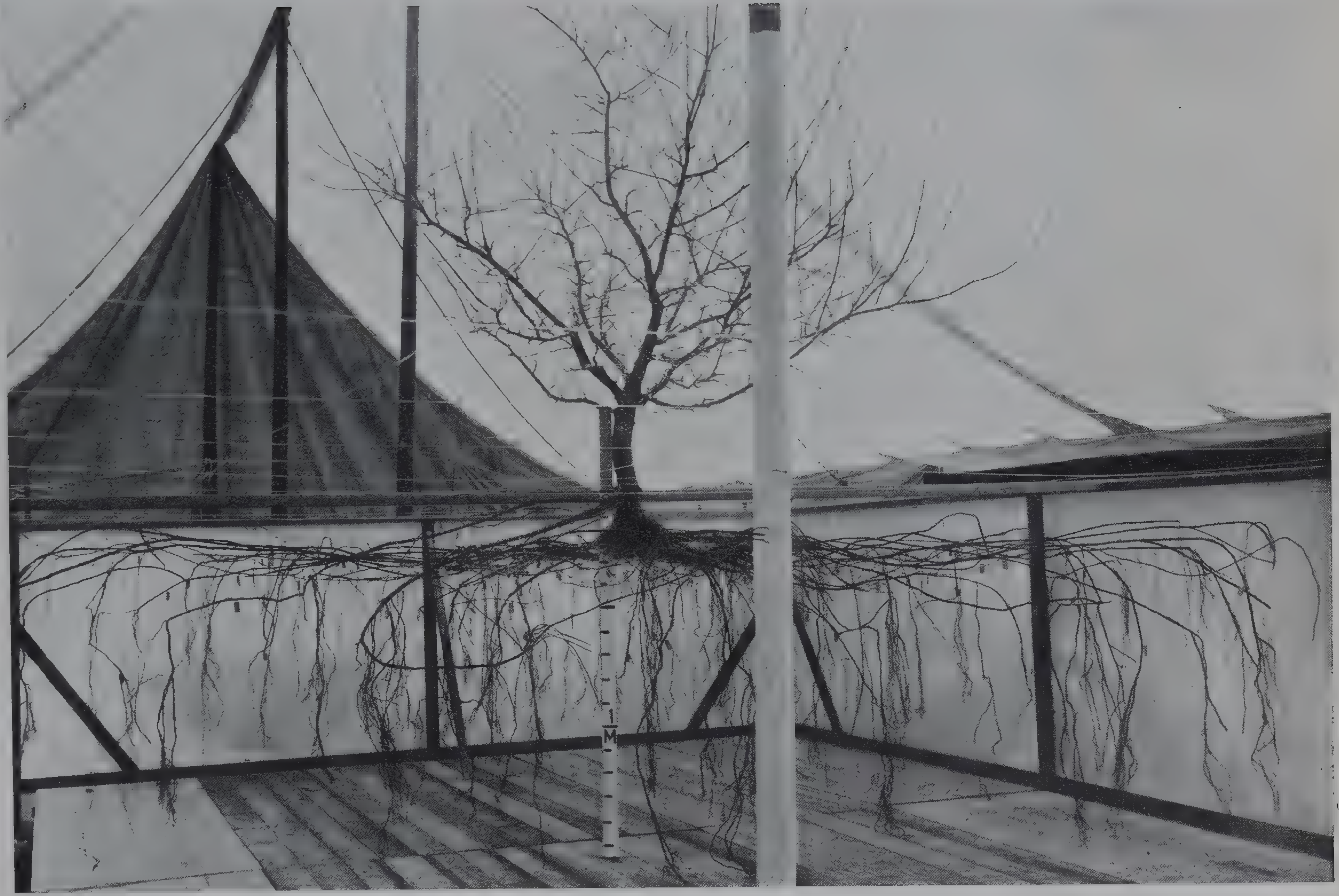
Ten year old Lane's Prince Albert apple on Stock No. II. Excavated from Sandy soil.

PLATE V.



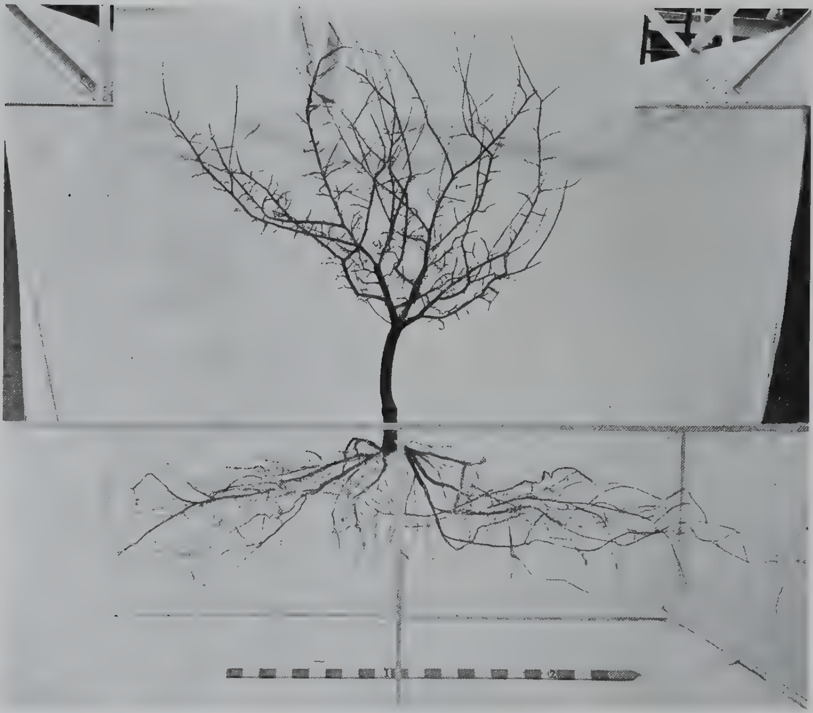
Ten year old Lane's Prince Albert apple on Stock No. I, excavated from Sandy soil.

Plate VI.



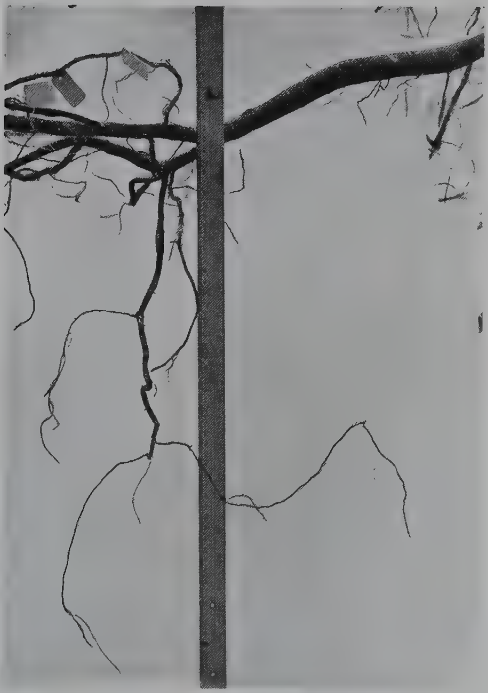
Ten year old Lane's Prince Albert apple on Stock O.F.5, excavated from Sandy soil.

PLATE VII.



Reconstructed root system of eleven year old Lane's Prince Albert apple on Stock No. II. Excavated from heavy Clay soil. The measure is marked in metres and tenths.

PLATE VIII.



Close view of root of Lane's Prince Albert on No. II on Clay soil.
Note the vertical root killed back.
Note also the rising laterals from the main root at about 50 cm. deep.

APPENDIX A.

Technique of Excavation, Grading and Weighing.

As already mentioned, the basis of our technique has been to dig a trench beyond the root area, and drive it in sections of 50 cm. across the ground occupied by the roots until the whole root system is excavated. The soil is gently removed in small pieces from the *side* of the wall, so that it falls away from the roots without breaking them, an ideal difficult but not impossible to attain with all but the very finest apple roots. In the method we called the "skeleton" system, as each root was carefully uncovered its position and depth was marked on a plan of squared paper, and it was then bent back out of the way or else labelled and detached. A complete plan of the roots was thus obtained, and from this plan the root system could afterwards be reconstructed, hanging in mid-air. The general conformation of the root system can then be studied, and photographs can be obtained.

Before starting the excavation the trunk of the tree was cut through, and the top was moved out of the way. For photographing it was joined on again with screws.

Another method was used to obtain accurate figures for the amount of root of various sizes. This we called the "block" method, for the soil was removed in blocks of known size and position, and all the roots from each block were placed in labelled bags, enclosed in an air-tight tin and despatched to the laboratory where they were washed, graded and weighed, as described below.

The size selected for a block was a 50 cm. cube, but the top cube was divided into three equal strata of about 17 cm. each (6½ inches), so as to give fuller information on the vertical distribution of the roots near the surface of the ground, where they were most abundant.

The arrangement of the blocks is shown in Fig. 1, which also shows how the bottom block might vary in size acording to the depth of the rock.

One drawback of this method is that where the roots of neighbouring trees interlace—as they did quite considerably at East Malling—some portions of root not belonging to the tree under excavation may be included with the correct roots, also some small roots may go beyond the excavation trench area and be missed. However, by tracing doubtful roots as far as possible before cutting off, it is hoped that these possible errors have been minimized.

The block method gives a picture of the root system in figures and in later excavations plans of the main roots were also obtained before the roots were removed from each block. No reconstruction is possible afterwards.

In the later excavations a "combined" method was used, which added to the skeleton method some of the advantages of the block method. At Wisley the trench was driven forward in 50 cm. sections as before. The roots were drawn on a plan but they were also cut and labelled at the boundaries of each block of 1 metre square and in two depths—above 30 cm. and below 30 cm.

The roots in each block could thus be grouped together and weighed, yet they could be joined together again later in their correct positions by the aid of the labels and root plan. It was not possible to subdivide the roots into grades when this method was employed. The roots were taken back to Malling, where they were washed and weighed in the laboratory.

The reconstructed root systems of some of these trees were exhibited at the Imperial Fruit Show at Birmingham in 1929 and at various other shows.

A further development of technique was employed at Wisboro' Green. Each root was cut and labelled at the boundary of every 50 cm. square and in four depths (0 to 30 cm., 30 to 50 cm., 50 to 100 and below 100), and was washed and weighed on the spot. The weighing was done with a battery of spring balances of various capacities suspended from a portable stand, which also served as a table for the root plan.

Thus the whole of the recording at Wisboro' Green was carried out in the trench.

Technique of Grading.

The roots of the Malling trees were washed free from soil in the laboratory, and divided into size grades with the aid of gauges. Seven grades were made in 1927-28, Grade I (or "fibre") consisted of root less than 1 mm. diameter; Grade II, root 1 to 2 mm. diameter; Grade III, roots 2 to 4 mm. diameter; and so on in geometric series. As it was found that the proportions of the higher grade roots varied considerably within a given stock, the number of grades was cut down to three in 1928-29, viz., below 1 mm., 1 to 4 mm. and over 4 mm.

The size gauges used, and the recorders operating them, were different in the two seasons 1927-28 and 1928-29, so an attempt was made to calibrate the two gauges and the personal factors of the recorders in the following manner. The roots from each of twenty-six blocks were graded by the 1928-29 recorders with the slotted gauges used that year, and the weights of each grade in each block determined in the usual way. All the roots from each block were then remoistened and thoroughly mixed up and regraded with the 1927-28 "peg" gauge by the recorder who had been responsible for the grading that year. The values for Grade I roots found with the 1927-28 grader were 9·3 per cent. less than those found with the 1928-29 grader, with a Standard Error of 1·8.

Weighing.

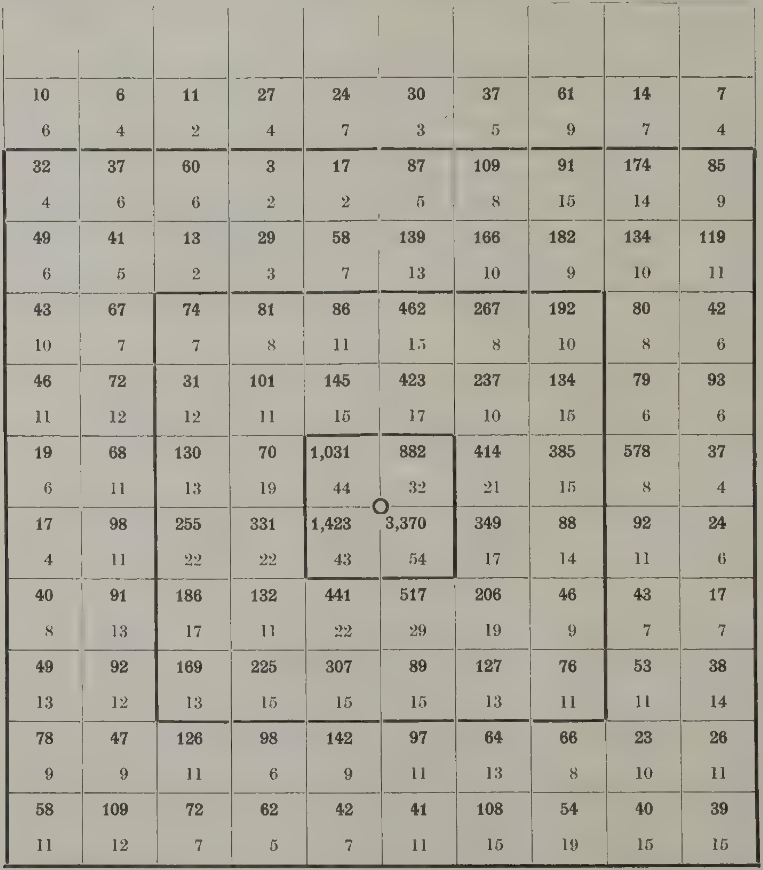
The fresh weight of each grade of root for each block was determined to the nearest ·1 gm. Although the advantages of dry weight determinations were fully recognized, fresh weights had to be used owing to the practial impossibility of drying at a constant temperature the large masses of material involved. The number of separate bags of roots (each from a single block of soil) sent in during the excavation of a single tree amounted sometimes to over 600, and, after subdivision into grades, the number of parcels of roots ran into several thousand. Moreover, all the records of the weight of stems, of prunings and of fruit with which the root weights would have to be compared, were on the fresh weight basis.

In so far as possible, the danger of errors due to different degrees of dryness was met by taking care that the roots were all in approximately the same condition when weighed. In the course of the process of calibration of the graders mentioned above, the total weight of roots in each of twenty-six blocks was determined twice, the roots having been remoistened and regraded between the two determinations. The second determination was sometimes slightly higher or slightly lower than the first, but the mean percentage difference between the pairs of values was only 0·115 with a Standard Error of 0·42, a surprisingly low value, showing that considerable success had been attained in maintaining uniform moisture conditions.

APPENDIX B.

Horizontal Spread of Roots.

The small squares represent columns of soil 50 cm. square in cross section, extending vertically down from the surface of the ground to the solid rock. The Upper value in each square (Clarendon type) denotes the total weight, in grammes, of roots found in that column. The Lower value denotes the number of grammes of fibre (roots less than 1 mm. diam.) in the column. The large squares correspond to the squares in Fig. 4. The small circle denotes the trunk of the tree.



10 6	**6** 4	**11** 2	**27** 4	**24** 7	**30** 3	**37** 5	**61** 9	**14** 7	**7** 4
32 4	**37** 6	**60** 6	**3** 2	**17** 2	**87** 5	**109** 8	**91** 15	**174** 14	**85** 9
49 6	**41** 5	**13** 2	**29** 3	**58** 7	**139** 13	**166** 10	**182** 9	**134** 10	**119** 11
43 10	**67** 7	**74** 7	**81** 8	**86** 11	**462** 15	**267** 8	**192** 10	**80** 8	**42** 6
46 11	**72** 12	**31** 12	**101** 11	**145** 15	**423** 17	**237** 10	**134** 15	**79** 6	**93** 6
19 6	**68** 11	**130** 13	**70** 19	**1,031** 44	**882** 32	**414** 21	**385** 15	**578** 8	**37** 4
17 4	**98** 11	**255** 22	**331** 22	**1,423** 43	**3,370** 54	**349** 17	**88** 14	**92** 11	**24** 6
40 8	**91** 13	**186** 17	**132** 11	**441** 22	**517** 29	**206** 19	**46** 9	**43** 7	**17** 7
49 13	**92** 12	**169** 13	**225** 15	**307** 15	**89** 15	**127** 13	**76** 11	**53** 11	**38** 14
78 9	**47** 9	**126** 11	**98** 6	**142** 9	**97** 11	**64** 13	**66** 8	**23** 10	**26** 11
58 11	**109** 12	**72** 7	**62** 5	**42** 7	**41** 11	**108** 15	**54** 19	**40** 15	**39** 15

(a) Root Distribution of No. I at Malling. Tree 1·5. 11 Years Old.

	15 8	**16** 7	**30** 9	**39** 4	**16** 3	**35** 4	**6** 3	**9** 5	**15** 3
	27 9	**34** 5	**54** 12	**87** 6	**66** 6	**16** 5	**44** 6	**7** 3	**13** 3
	45 11	**78** 9	**221** 7	**96** 7	**131** 7	**105** 9	**50** 4	**45** 10	**55** 10
	24 8	**40** 12	**135** 10	**191** 7	**288** 7	**113** 7	**81** 4	**88** 8	**16** 6
	16 7	**28** 10	**126** 8	**253** 8	**385** 6	**205** 10	**151** 7	**70** 8	**45** 8
	24 9	**40** 12	**140** 15	**713** 20	**1,332** 10	**630** 12	**123** 7	**72** 10	**43** 11
	51 16	**107** 15	**585** 19	**1,015** 16	**2,228** 15	**110** 10	**125** 12	**38** 12	**43** 19
	68 9	**142** 19	**170** 12	**213** 5	**334** 10	**142** 10	**137** 11	**112** 14	**49** 14
	68 14	**100** 7	**113** 6	**155** 7	**62** 9	**295** 12	**227** 6	**61** 12	**30** 15
	54 12	**39** 10	**110** 13	**69** 11	**49** 9	**84** 10	**38** 8	**119** 11	**49** 15
	36 14	**54** 13	**51** 13	**53** 8	**39** 9	**85** 16	**25** 8	**35** 13	**60** 17

(b) ROOT DISTRIBUTION OF NO. II AT MALLING. TREE 1·6. 11 YEARS OLD. Figures in heavy type show total weight of roots in square indicated, figures in light type show weight of fibre.

14	**14**	**27**	**44**	**25**	**46**	**11**	**24**	**44**	**27**	**14**	**9**
7	11	12	11	8	6	5	5	2	6	6	4
13	**19**	**63**	**43**	**87**	**72**	**41**	**108**	**66**	**50**	**38**	**34**
6	12	13	15	12	18	8	11	6	7	7	11
30	**57**	**72**	**123**	**165**	**166**	**70**	**173**	**108**	**163**	**71**	**41**
18	21	19	21	20	27	12	12	8	10	11	8
22	**53**	**72**	**136**	**246**	**429**	**188**	**438**	**123**	**101**	**23**	**7**
16	21	24	26	23	23	18	18	8	12	9	3
34	**81**	**97**	**205**	**164**	**382**	**1,217**	**774**	**194**	**58**	**72**	**15**
11	21	17	22	13	25	15	28	8	11	11	9
50	**93**	**124**	**185**	**558**	**1,980**	**3,836**	**183**	**126**	**110**	**63**	**21**
12	20	20	15	25	20	20	19	9	12	10	8
39	**86**	**178**	**323**	**500**	**3,701**	**2,304**	**428**	**95**	**86**	**20**	**19**
13	19	24	19	20	25	19	15	11	10	11	7
51	**60**	**66**	**167**	**442**	**404**	**1,856**	**216**	**179**	**157**	**62**	**17**
14	20	26	21	22	14	21	12	9	12	13	6
45	**68**	**75**	**279**	**482**	**211**	**288**	**190**	**121**	**182**	**45**	**22**
17	24	22	22	24	12	16	26	8	8	8	7
33	**57**	**75**	**137**	**166**	**93**	**120**	**128**	**135**	**58**	**25**	**12**
10	20	17	20	17	13	11	13	15	10	10	7
40	**63**	**66**	**76**	**93**	**40**	**59**	**65**	**62**	**43**	**17**	**14**
15	21	22	18	14	14	15	11	6	6	9	6
9	**18**	**15**	**17**	**5**	**4**	**16**	**27**	**26**	**21**	**10**	**3**
6	10	7	7	2	1	5	2	8	6	7	2

(c) Root Distribution of No. XVI at Malling. Tree 14·2. 11 Years Old. Figures in heavy type show total weight of roots in square indicated, figures in light type show weight of fibre.

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INJECTION OF FRUIT TREES: PRELIMINARY EXPERIMENTS WITH ARTIFICIAL MANURES

By LEONARD A. THOMAS
Council for Scientific and Industrial Research, Australia

AND

W. A. ROACH
Research Station, East Malling

INTRODUCTION.

WITHIN the last few years much knowledge has been gained concerning the so-called deficiency diseases of fruit trees and the recognition of their symptoms in the orchard. Wallace (14) has carried out extensive work with apple trees in sand cultures and the deficiency symptoms for all the major elements used as plant foods have been established. The obvious practice for overcoming these deficiencies is to apply artificial manures, which contain the necessary elements, to the soil. In most cases this has been done with success, but the application of artificial manures, even in large amounts, is not always effective in a reasonable length of time. Thus Wallace (15) endeavoured to remedy potassium deficiency in red currants by applying potassium sulphate to the soil. After two seasons of application (4 cwt. per acre per annum) there was no appreciable decrease in the trouble. He suggested that the potash became "locked-up" in the soil. Similar cases of lack of response to potash manuring, even though a deficiency of this substance was suspected, have been recorded in America (4).

A method of applying the manure which would give a more rapid and certain response would, therefore, be valuable. Direct injection of suitable salts into the trees suggests itself as a possibility.

The extensive literature of tree injection has been reviewed by others (2, 6); detailed reference to it here is therefore unnecessary. Most of this literature concerns attempts to cure insect and fungus diseases but a few attempts have been made to cure deficiency diseases by this method (1, 10, 11). Few, if any, of the methods so far employed are ideal for extensive use; the ideal method should be simple and rapid and the apparatus employed should be inexpensive; physical and systemic damage suffered by the tree must be reduced to a minimum; and the resulting distribution of the injected substance must be accurately controlled.

During the last few years Roach (7, 8, 9) has carried out experiments to simplify and cheapen the technique and to trace the course of distribution of the injected liquids under different circumstances. As a result of these experiments, it is now possible to inject a liquid with precision:

(i.) so that it is distributed uniformly throughout the whole tree;

(ii.) so that two or more main branches of a single tree, with their associated roots, may be injected for comparative purposes, each with a different liquid without any intermixing of the injected substances;

(iii.) so that smaller branches ranging in size down to current year's shoots may each be independently injected with different substances without these substances reaching the roots or intermixing.

The last two methods open up the possibility of actually ascertaining in the plantation itself what manure is lacking, by experiments on a small number of trees. To be able to do this is highly desirable for two reasons: first, because leaf, or other, symptoms do not always give a clue as to what manure is needed; and, second, because visual proof that a cure would eventually result from the application of such a slow acting manure as potash would encourage a grower to persevere with the treatment. Having ascertained what is wanted, the first method may well prove to be an economical way of treating the whole plantation for certain deficiencies which are not readily dealt with by ordinary manurial treatment.

These experiments, in common with those recorded in the literature, were sometimes, but not always, followed by considerable damage to the trees; and it was realized that however convenient and hopeful the injection method might be, it would be quite useless as a practical method unless all serious damage could be avoided. The following experiments were therefore carried out mainly to study this question, but also to make preliminary observations on the nutritional effects of the various substances injected.

They were devised (1) to give a general survey of the problem by injecting solutions of the compounds commonly used as manures into two varieties of apple trees, each worked on three different rootstocks; this should give a measure of varietal and individual differences in response to the various compounds; (2) to ascertain how far the damage was due to the use of salts in a physiologically unbalanced condition; and, (3), to determine how the damage was influenced by the absolute amount of substance injected, and by the concentration of the solution.

EXPERIMENTAL MATERIAL.*

The trees experimented upon were of two varieties: Beauty of Bath and Cox's Orange Pippin, each worked (the majority budded, the rest grafted) on three different rootstocks, Malling Nos. I, V and XII. At the end of the first growing season following the budding or grafting, the single stems had been shortened to a uniform height of 2 ft. above ground level; and during the following season the top four or more buds had grown into branches. Normally the trees would have been removed from the nursery at this stage and spaced more widely in the field (they stood about 1 foot apart in rows, which were 3 ft. apart); for these experiments, however, it seemed preferable to risk the effects of overcrowding rather than transplant the trees just prior to the first injection.

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* Much of this material was originally raised for the Research Department of Imperial Chemical Industries to whom best thanks are due for releasing the trees for these experiments.

In November 1932, the percentage of leaves on each tree showing leaf scorch, of potassium deficiency type, was estimated; and in December the total length of wood formed during the current growing season was measured.

Grading.

The trees were arranged into experimental units of 5 or 4 trees in such a way that each unit contained, as far as possible, trees of the same range of size, the same average size and the same percentage of leaf scorch.

The trees were winter pruned to give four leaders for future growth.

Pruning.

Approximately two thirds of the wood was removed from each tree, according to the practice adopted at East Malling. This meant that a long leader had a little more than two thirds of its wood removed, while a short leader had a little less than two thirds removed. In this way the tree was "evened-up". All lateral growths were removed from the leaders and all shoots arising from the trunks of the trees; this latter practice was carried out in the ensuing growing season so as to confine growth to the leaders.

Spraying.

Routine spraying was carried out for the control of insect and fungus pests.

*Method of Injecting the Solutions.**

In these experiments small trees were injected through a single hole in the main stem to give as uniform distribution as possible of the injected substance throughout the whole tree.

For this purpose a simple form of apparatus was used, as illustrated in Fig. 1. At a selected point on the main stem, about twelve inches above ground level, a piece of bark was removed with a cork borer $\frac{1}{4}$ inch in diameter. A single hole $\frac{1}{8}$ inch in diameter was bored centrally at this point through the tree. The glass nipple of the apparatus was inserted so as just to touch the wood, and the clamps were screwed into position. The solution was then introduced into the tube, and the air carefully released from the bore-hole without loss of solution. This was done by slightly unscrewing the clamps. No great pressure was exerted by the thumb screws, as undue pressure caused the bark to split, particularly during early Spring when the cambium was active. The holes were orientated to give, as far as possible, equivalent amounts of solution to each branch, as explained by one of us elsewhere (8, 9).

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* Since these experiments were carried out, a simpler and quicker method has been devised (9).

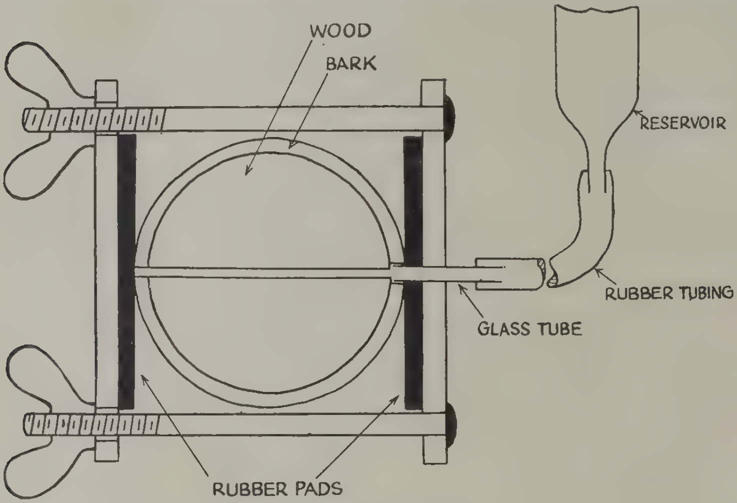

FIG. 1.
Apparatus used for tree injection.

SUBSTANCES INJECTED.

A list of the substances injected will be found in Table I.

Amounts Injected.

It will be seen that the amounts of the various single salts were so arranged that, as far as possible, a given element or radicle was injected in constant amount in its various compounds, e.g., whenever potassium was injected its amount was 0·145 gm.; the phosphate radicle varied in amount, because commercially important phosphates (potassium monohydrogen phosphate and ammonium dihydrogen phosphate) were used in preference to the chemically most nearly related ones; it was not realized at the time that diammonium phosphate is manufactured in large commercial quantities. Enough of these compounds was given to supply the standard amounts of potassium and nitrogen.

The mixed salt solutions (the 2 salts and 3 salts) were made up by mixing equal volumes of the solutions of the constituent salts. In this way the osmotic

TABLE I.

Damage caused by

Substance.	Amount per tree. gm.	*Winter Injection.* B.B./XII.	C.O.P./XII.	B.B./V.	C.O.P./V.	*Summer Injection.* B.B./XII.	C.O.P./XII.	B.B./V.	C.O.P./V.	B.B./I.	C.O.P./I.
Potassium nitrate	$\frac{[KNO_3]}{200}$ = 0·505	00000	00000	**DDDD** Bk	00000	**L** LLL	LLLL	**LLL** LL	**LLLLL**	**L** LLL	LLLL
Potassium phosphate	$\frac{[K_2HPO_4]}{400}$ = 0·435	00000	00000	00000	00000	L 000	LL00	00000	00000	0000	LLLL
Potassium sulphate	$\frac{[K_2SO_4]}{400}$ = 0·436	00000	00000	00000	00000	0000	0000	00000	00000	**L** L 00	**LL** LL
Potassium chloride	$\frac{[Kcl]}{200}$ = 0·373	00000	00000	**DD** 000	00000	LLLL	**L** LLL	LL 000	**L** LLLL	LLLL	**LLLL**
Sodium nitrate	$\frac{[NaNO_3]}{200}$ = 0·345			**D** Bk Bk 00	00000			**L** L 000	**LLLLL**		
Sodium sulphate	$\frac{[Na_2SO_4 + 10H_2O]}{400}$ = 0·806			00000	00000			L 0000	00000		
Magnesium nitrate	$\frac{[Mg(NO_3)_2 + 6H_2O]}{400}$ = 0·641			BkBkBkBk0	00000			**L** L 000	**LL** LLL	LLLL	**LLL** L
Magnesium sulphate	$\frac{[MgSO_4 + 4H_2O]}{400}$ = 0·557			00000	00000			00000	**LL** LLL	**L** LLL	**LL** LL
Magnesium chloride	$\frac{[MgCl_2 + 6H_2O]}{400}$ = 0·508									LLLL	**L** LLL
Calcium nitrate	$\frac{[Ca(NO_3) \times 2H_2O]}{400}$ = 0·197			00000	00000			**LLLLL**	**LL** LLL		
Calcium chloride	$\frac{[CaCl_2 + 6H_2O]}{400}$ = 0·550	00000	00000	00000	BdBd000	**L** LLL	**LL** LL	LLLLL	**LLL** LL	**L** LLL	**LL** LL

*Potassium sulphate	$\frac{[K_2SO_4]}{800}$	$=0\cdot218$	ooooo	ooooo	ooooo	ooooo	ooo	ooo	ooooo	ooooo	oooo	LLLL
Ammonium phosphate	$\frac{[NH_4H_2PO_4]}{400}$	$=0\cdot288$										
†Calcium nitrate	$\frac{[Ca(NO_3)_2]}{1200}$	$=0\cdot197$			ooooo	ooooo			ooooo	ooooo		
Magnesium sulphate	$\frac{[MgSO_4+4H_2O]}{1200}$	$=0\cdot186$										
Potassium phosphate	$\frac{[K_2HPO_4]}{1200}$	$=0\cdot218$										
Ammonium nitrate	$\frac{[NH_4NO_3]}{400}$	$=0\cdot160$	ooooo	ooooo	**DD** Bk Bk o	ooooo	**LLL** L	**LLLL**	**L** Bk ooo	**LLL** LL	**LL** LL	**LLLL**
Ammonium phosphate	$\frac{[NH_4H_2PO_4]}{200}$	$=0\cdot575$	ooooo	ooooo	ooooo	ooooo	oooo	oooo	L oooo	oooo	LLLL	LLLL
Ammonium sulphate	$\frac{[(NH_4)_2SO_4]}{400}$	$=0\cdot330$	ooooo	ooooo	ooooo	Bk oooo	**LLLL**	**LLLL**	**LLLLL**	**LLLLL**	**LL** LL	**LLLL**
Ammonium chloride	$\frac{[NH_4Cl]}{200}$	$=0\cdot267$			ooooo	Bk oooo			ooooo	**LLL** LL		
Urea	$\frac{[CO(NH_2)_2]}{400}$	$=0\cdot150$			ooooo	ooooo			oooo	ooooo	oooo	oooo
Date of Injection			24–27/3/33	27–30/3/33	12–23/3/33	1–12/3/33	29–30/5/33	30–31/5/33	22–29/5/33	15–18/5/33	31/5–1/6	1–2/6/33

* Referred to for brevity as two salts.
† Referred to for brevity as three salts.
Rootstock types in Roman numerals.
L=Tree with leaves heavily damaged.
L=Tree with leaves lightly damaged.

B.B.=Beauty of Bath.
C.O.P.=Cox's Orange Pippin.

D=Dead tree.
D=Tree dead above injection hole.
Bk=Tree with some dead bark.
Bd=Tree with some dead buds.
o=Undamaged tree.

concentration remained the same as that of the single salt solutions, but the amount of each constituent salt was less than in the single salt solutions.

In deciding on the actual amount to be injected, the ideal aimed at was to produce damage with at least some of the substances, but to get increased growth and vigour in spite of the damage. It will be seen that the first part of this ideal was, in fact, attained and that, in spite of the conditions already mentioned (page 153) militating against the attainment of the second part of the ideal it also was attained to some extent.

Concentration.

For the winter injections, the required amount of substance was dissolved in 25 cc. water, this being taken up in two to three days; but in Summer it was dissolved in 50 cc.; this larger volume being absorbed in about one hour. The concentration of the injected solution was thus N/5 in Winter and N/10 in Summer.

DAMAGE.

The healing of the injection holes was apparent a few weeks after the injection and almost every hole was completely healed by the end of the growing season, including those of trees on which excessive pressure on the clamp had caused the bark to " slip ".

In the Winter following the injections, a number of trees injected with various salts were sectioned to study the injury suffered by the wood. A section through the hole showed a narrow brown border (never more than a millimetre deep) on either side of the hole; and the ends of each hole were seen to be healed in a normal way. Sections above and below the hole demonstrated a brown " shadow " of the hole and " borders ", both becoming lighter in colour the greater the distance from the hole. Usually this browning could only be traced 5 cm. above and below the hole, but in a few cases it reached 15 cm. In every tree examined the wood formed subsequent to the injection was healthy.

There was no evidence of any microbial invasion, whether the hole was plugged or left open.

Damage caused by Winter Injection.

The damage caused by injection of salts in Winter became aparent at bud break and extended gradually for some weeks. Four trees were actually killed; some had strips of bark killed, these strips usually beginning at the hole, and others had buds killed. This damage is recorded in Table I.

All the four trees actually killed were of the variety Beauty of Bath on rootstock V (B.B./V), as also were fourteen trees out of a total of eighteen which were damaged but not killed; trees of variety Cox's Orange Pippin on the same rootstock and of about equal size had none killed and only four trees damaged. These facts suggest a varietal difference in susceptibility to injection damage.

None of the trees of either variety on rootstock type XII showed any damage. The results for similarly injected trees on the two rootstocks were as follows:

TABLE II.

Effect of Variety and of Rootstock on Amount of Damage.

	B.B./V.	B.B./XII.	C.O.P./V.	C.O.P./XII.
No. of trees killed	3	0	0	0
No of trees damaged but not killed	8	0	3	0
No. of trees undamaged ..	34	45	42	45

That the greater average size of the trees on rootstock XII as compared with those on V was not responsible for these facts is apparent for two reasons: first, when similarly injected trees of the same variety on the same rootstock are considered, size has little or no effect on the amount of damage; and second, when trees of similar size are considered, the greater damage suffered by trees on V is still apparent. In Table III trees injected with potassium nitrate and ammonium nitrate respectively are arranged in order (1) of trunk volume and (2) of current year's shoot growth; these arrangements should correspond sufficiently closely with an arrangement strictly in order of size. The deduction drawn from this table, viz.: that the trees on type XII did not escape damage because of their greater average size, was also verified for all the other salts injected. The chances of all the eleven damaged B.B. trees being distributed by accident among those trees on one rootstock is 1 in 2^{10} or 1 in 1,024. The facts therefore suggest that a tree's susceptibility to injection damage is influenced by the rootstock on which it is worked. It cannot, of course, be assumed, on the basis of one season's results, that this influence will always show itself in the same way; but the results do show that it is unsafe to assume that a solution found innocuous when injected into trees of one variety on one rootstock will also be innocuous when injected into trees of another variety on the same rootstock, or into trees of the same variety on another rootstock.

Hafekost's work on the "wilting limits" of different scion and rootstock varieties is of interest in this connection (3). The most striking fact revealed by a comparison of the relative amounts of damage caused by the various substances is the high toxicity of nitrates to B.B./V. Of the twenty-two damaged trees no less than sixteen were injected with nitrates. Potassium,

TABLE III.

Effect of Rootstock on Amount of Damage.

Salt.	Volume of Trunk, ml.	Stock XII.	Stock V.
KNO_3	185	None	Bark damaged
	150		Dead
	147		Dead
	143		Dead
	137		Dead
	75	None	
NH_4NO_3	232		Bark damaged
	224	None	
	213	None	
	188		Dead
	185	None	
	173		Partly dead
	156		Dead
	145	None	
	Shoot Length, cm.		
KNO_3	450		Bark damaged
	393	None	
	353	None	
	339		Dead
	307		Dead
	301	None	
NH_4NO_3	381		Bark damaged
	380	None	
	325	None	
	324		Dead
	293		Partly dead
	262		Dead
	253	None	

sodium, magnesium and ammonium nitrates damaged five, three, four and four trees respectively; calcium nitrate, however, caused no detectable damage. The damage caused by nitrates was confined entirely to B.B./V, a similar series of C.O.P./V trees being undamaged. Potassium and ammonium nitrates, the only nitrates injected into trees on XII, caused no damage to either variety on this rootstock, whereas they caused serious damage to trees of B.B./V. Further reference will be made later to the comparatively high toxicity of the nitrate form of nitrogen when injected.

Damage caused by Summer Injection.

The damage resulting from the summer injections became apparent within a few hours of the injection, and reached its maximum within twenty-four hours. It differed markedly from that produced by winter injections; whereas the winter injection damaged the bark and buds and, in extreme cases, whole branches or even the whole top of the tree, the summer injections damaged the leaves only (only one example each of bark damage and death of a branch occurred among 209 damaged trees). This damage was confined entirely to the older leaves; those borne on the unlignified terminations of the shoots showed no damage.

All salts except ammonium sulphate and ammonium nitrate caused a similar type of damage; most damaged leaves had their edges scorched, more severely damaged ones had dead brown areas between the lateral veins, and the edge-damage extended towards the midrib between the lateral veins. Leaves which were only edge-damaged were indistinguishable from those showing leaf scorch associated with potash deficiency.

Ammonium sulphate caused the death of an entire part of a leaf or the complete leaf was killed; usually some defoliation took place within a week after the injection. Similar damage was caused by solutions of ammonium nitrate, but it was less severe and no leaves fell from the trees. The severity of the damage caused by the various salts is indicated in Table I. No marked effect either of variety or rootstock was apparent; but the amount of damage differed according to the substance injected. In general, substances causing no damage when injected in Winter caused little damage when injected in Summer, but this parallelism was not a close one; ammonium sulphate was an exception, since it caused comparatively little damage in Winter and much in Summer. Urea was the only substance causing no damage when injected alone.

The distribution of the damage shown in Table I suggests that most of the damage resulting from winter injection and a certain amount of that resulting from summer injection could have been avoided by suitable choice of compounds containing the elements to be injected, nitrogen being by far the most important element in this respect; but there still remains a considerable amount of damage which cannot be avoided in this way, resulting particularly from summer injection. Some other way of lessening this must be found.

ANTAGONISM.

It is well known that the various mineral elements required by the plant must be supplied in certain fairly definite relative amounts if the plant is to make optimum healthy growth; and that when any one of them is supplied as a pure salt, it is invariably toxic to the plant (5). The work of Osterhout and others on the phenomenon of antagonism, i.e. the neutralization of the toxicities of pure salts when they are mixed in the right proportions, is too well known to need detailed reference. The full toxicity resulting from the absorption of a pure salt in a water culture experiment is unlikely to result from its injection into a growing tree, since other salts are at the same time being absorbed through the roots; but a lack of balance between the salt constituents of the tree might well result from such an injection. If this were so, injection damage might be lessened by suitable "blending" of the cations. Potassium was selected for study since it is more often necessary to apply it to fruit soils than any other element; and calcium was selected as a convenient antagonizing element.

Preliminary Experiment on Dormant Shoots.

A preliminary experiment was carried out in the Winter on dormant current year's shoots (winter prunings). Shoots of equal size were put each with its cut end under 10 cc. of solution in a glass tube. The solutions were made by mixing N/5 solutions of potassium nitrate and calcium nitrate in the following ratios: 10:0, 9·9:0·1, 9:1, 4:1, 1·5:1, 1:1·5, 1:4, 1:9. Four shoots were used for each ratio. The shoots in the 4:1 ratio solution both lived longest and produced the best developed leaves; the length of life and the leaf development fell off in the other solutions as the ratio diverged from this optimum 4:1 one.

Summer Injection.

An actual injection experiment was carried out in the Summer on Beauty of Bath trees on rootstock type I. Five trees constituted a unit for each ratio and the trees were selected so that all the units contained trees of equal average size and of the same range of size. N/10 solutions were used in the same ratios as in the above preliminary experiment.

The damage, which was confined to the leaves, was estimated by counting the number of leaves with ¼, ½, ¾ and the whole of their area injured and expressing the total damaged area as a percentage of the total leaf area of the tree. The results are shown graphically in Fig. 2 in which the percentage leaf

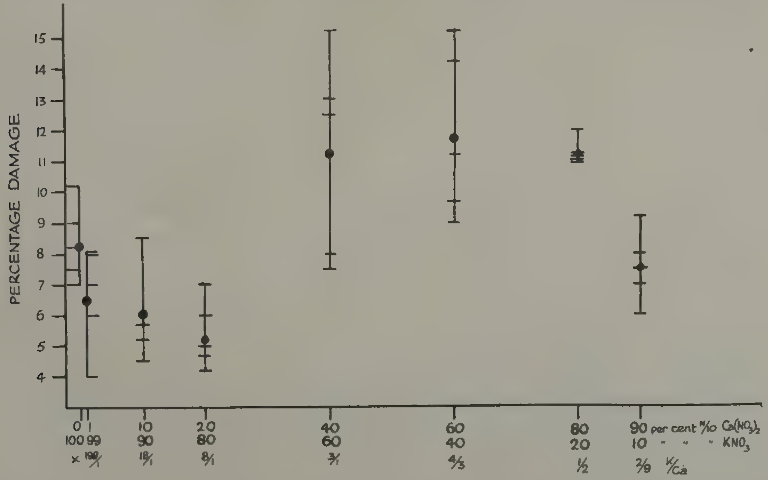

FIG. 2.

Damage caused by various mixtures of calcium nitrate and potassium nitrate solutions.

damage for each tree is plotted separately as a short horizontal line, and the average percentage leaf damage for each solution ratio is represented by a black point.

As the ratio of calcium to potassium was increased, the leaf damage decreased until a solution ratio of $4KNO_3 : 1Ca(NO_3)_2$ was reached, after which it increased rapidly. Visual examination of the trees led to this same conclusion. This optimum solution ratio is the same as that obtained in the first experiment and corresponds to a K/Ca ratio of 8:1.

Another experiment was carried out in the same manner as the above, using calcium to antagonize magnesium. The nitrates were again used in N/10 solutions. Neither the data obtained from the percentage of leaves damaged nor from visual observation gave any definite result.

The antagonism between calcium and magnesium is rather weak, as these two cations lie near to each other in the lyotropic series, hence this method of studying the effects of antagonism of ions appears to be unsuitable for weakly antagonistic ions.

Another series of Beauty of Bath trees on Stock I were treated with the following amounts of N/10 solutions in an attempt to reduce further the damage caused to the trees by employing the ions of $K^{\cdot}$, $Na^{\cdot}$, $Mg^{\cdot\cdot}$ and $Ca^{\cdot\cdot}$.

Treatment.

1. 40 cc. KNO_3 + 10 cc. $Ca(NO_3)_2$.
2. 40 cc. ,, + 5 cc. ,, + 5 cc. $Mg(NO_3)_2$.
3. 30 cc. ,, + 10 cc. ,, + 10 cc. ,,
4. 30 cc. ,, + 5 cc. ,, + 15 cc. ,,
5. 30 cc. ,, + 10 cc. ,, + 10 cc. $NaNO_3$.
6. 30 cc. ,, + 5 cc. ,, + 5 cc. $Mg(NO_3)_2$ + 10 cc. $NaNO_3$.
7. 20 cc. ,, + 5 cc. ,, + 5 cc. $Mg(NO_3)_2$ + 20 cc. $NaNO_3$.
8. 12·5 cc. ,, + 12·5 cc. ,, + 12·5 cc. $Mg(NO_3)_2$ + 12·5 cc. $NaNO_3$.
9. 12·5 cc. ,, + 12·5 cc. ,, + 12·5 cc. $Mg(NO_3)_2$ + 12·5 cc. $NaNO_3$.
10. 12·5 cc. ,, + 12·5 cc. ,, + 12·5 cc. $Mg(NO_3)_2$ + 12·5 cc. $NaNO_3$.

Four trees constituted a unit.

Treatment 7 was the most successful, followed by treatment 8; all the other treatments appeared to produce about the same degree of damage, thus it was possible to reduce further the damage caused by the most favourable combination of the ions of potassium and calcium previously found by the use of a solution containing the ions of $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$ and $Na^{\cdot}$.

The fact that the phenomena of antagonism, first observed in animal transfusion experiments and later in plants growing in water culture etc., are also observed when the solutions are injected into trees, is interesting: but the lessening of injection damage brought about is insufficient to make it of practical value in injecting the nutrient elements mentioned.

EFFECT ON DAMAGE OF CONCENTRATION OF SOLUTION AND OF TOTAL QUANTITY OF SUBSTANCE INJECTED.

Trees of Cox's Orange Pippin on rootstock I of approximately equal size were injected in the Summer with three different amounts of potassium nitrate each in a number of different volumes of solution. The resulting damage is indicated graphically in Fig. 3. Each observation represented was obtained from at least two trees. As a result of exercising care in orientating the injection holes, the distribution of the damage was uniform over the whole of each tree. Similarly treated trees were damaged to the same extent.

In the diagram the severity of the damage is represented by the varying diameter of the dead black circles, lack of damage being indicated by open circles. A smooth curve is drawn through the smallest dead black circles, i.e. through points representing volume-amounts causing just detectable damage. Below and to the right of this line are larger circles representing the more serious damage resulting from larger amounts or greater concentrations than those represented by points on the curve. The open circles to the left of and above the curve represent lower volume-amounts which cause no damage.

In order to verify these results the injections which caused just detectable damage, i.e. those represented by points on the line, were repeated on at least two further trees for each volume-amount; the resulting damage was identical in every case. Further replication, therefore, appeared unnecessary; the chances of obtaining by accident the above perfectly regular distribution of results are extremely remote.

It will be seen that $\frac{1}{4}$ gm. when injected in 25 cc. caused just detectable damage, but was harmless when injected in 50 cc.; $\frac{1}{2}$ gm. caused serious damage when injected in 50 cc. of solution and slight damage in 100 cc., i.e. $\frac{1}{4}$ gm. injected in 25 cc. causes the same amount of damage as $\frac{1}{2}$ gm. in 100 cc. Equal damage was also caused by 1 gm. in 400 cc. Hence to produce equal damage the volume of solution must increase as the square of the amount of substance.

Probably the above facts are explicable in terms of the rate at which the leaf cells are able to remove salts from the "injection stream" and translocate them to other parts of the tree and metabolize them. If salts be supplied in the injection stream at a greater rate than they can be dealt with by the leaf cells, they may easily reach toxic concentrations.

How far dilution of the injected solution can be used as a practical means of avoiding injection damage in Summer will depend on the quantities of the various salts necessary to produce optimum effects on the trees ; these quantities can only be determined by experiment, but there is no reason for supposing, at this stage, that damage is likely to limit seriously the application of injection as a commercial method, if applied in the Summer.

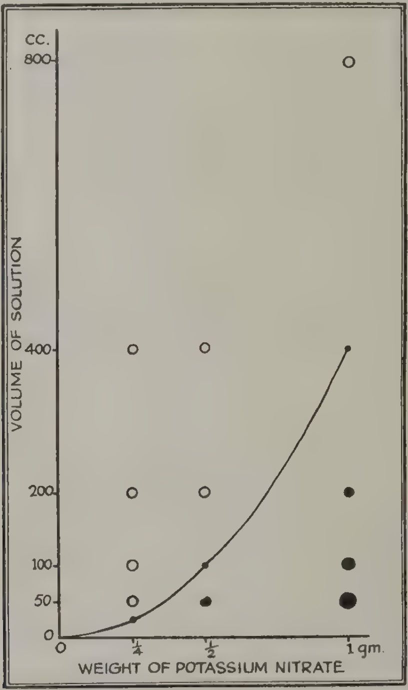

Fig. 3.

Damage caused by varying amounts of the same substance in varying volumes of solution.

Injection of Nitrogen Compounds.

The differences in degree of damage caused by injecting the same amount of nitrogen as nitrate, ammonium salt and urea has already been noticed. To get a clearer idea of the comparative toxicities of these compounds, equal sized trees were injected in the Summer in quadruplicate with 50 cc. of solution per tree. The data are given in Table IV.

The figures in the fourth column represent degrees of severity of damage, estimated by eye.

TABLE IV.

Damage caused by Different Nitrogen Compounds.

Compound.	Weight injected.	Proportionate amount N.	Damage for each tree.	Total damage.
Potassium nitrate	0·5 gm.	1	3, 3, 4, 4	14
Ammonium nitrate	0·8 gm.	2	4, 5, 4, 5	18
,, ,, ...	0·4 gm.	1	1, 1, 1, 2	5
Urea	0·15 gm.	1	0, 0, 0, 0	0
,, ...	0·3 gm.	2	0, 0, 0, 0	0
,, ...	0·45 gm.	3	1, 0, 1, 1	3

The above results confirm the conclusion already arrived at that when equal amounts of nitrogen are injected in the three forms, the toxicity decreases in the order: potassium nitrate, ammonium nitrate, urea. Further, the maximum safe dose of nitrogen in the form of urea is rather more than three times that in the form of ammonium nitrate and probably about six times that in the form of potassium nitrate. Such a result can only be partially explained in terms of the comparative osmotic pressures of the three isolations; probably the comparative rapidity with which the three compounds are absorbed from the injection stream into the cells and metabolized also plays a part.

This marked toxicity of nitrates when injected (see also pages 156, 158) is of particular interest in view of the following observations made by W. Thomas in regard to the distribution of nitrates in apple trees (12, 13): "The fine roots gave nitrate reactions throughout the season; whereas in the main roots the reaction was much feebler and . . . the tests were negative in the aerial parts except in the buds as they were opening." He states that the nitrates absorbed by the roots are normally rapidly transformed in the roots into other compounds and the rest of the tree, with the exception of the buds at the short period mentioned, are free from nitrate; moreover, judging by the behaviour of plant extracts, it is only in the fine roots that nitrate reduction can take place at all rapidly. The variation in this toxicity according to variety and rootstock is also of some interest in this connection.

These results are important from the present point of view, however, for two main reasons:

(1) they prove that the injection of a substance is not always followed by the same effects on the tree as those resulting from its absorption through the roots, and

(2) they indicate that greater care must be exercised in choosing the particular compound containing the element to be injected than is necessary when applying it to the soil or adding it to a water culture solution.

EFFECT ON GROWTH.

The trees were growing in rich soil and the growth made in the 1933 season was nearly the optimum for that type of material; any large response to differential supplementary nutrition was, therefore, unlikely. Moreover, there was practically no leaf scorch nor other visible signs of potash deficiency, even on the trees on type V, in 1933.

There were, however, indications of some increase in growth as a result of the injection of certain salts, particularly those containing potassium and nitrogen, into trees on type V, but for only one solution was the increase of real statistical significance. Whereas the extension growth made in 1933 by untreated and water injected trees was on the average 120 per cent. of that made by the same trees in 1932, the corresponding figure for the trees injected with a mixture of potassium sulphate and ammonium phosphate was 153 per cent. Miss J. L. Edgar, after a statistical analysis of the growth measurements, found that the odds were 100 to 1 against this difference of roughly 27 per cent. in growth being due to chance.

Further evidence of such increased growth and of improvement in health resulting from injection is supplied by experiments described in detail elsewhere (7, 8). In one of these experiments the injection of potassium nitrate solution into an apple tree doubled its extension growth and the leaves which expanded after the injection were thick, large and of a healthy dark green colour, in marked contrast with the thin sickly looking light green leaves borne by the tree at the time of the injection and in previous years. This marked increase in vigour of the whole tree persisted during the following season, at the end of which the tree was felled.

In two other experiments apple mildew was controlled as a result of an injection of a dilute solution of sodium thiosulphate. In one of these there was a marked increase in vigour also.

A number of such beneficial effects have been recorded in the literature, for example, Storey and Leach (10) have recently detected and cured a sulphur deficiency disease of the tea bush by solution injection experiments, and the treatment of trees suffering from lime-induced chlorosis by the solid and solution injection methods described by Bennett (1) is now a widely used commercial method.

There is every hope, therefore, that by using suitable methods of injection and correct concentrations and quantities, deficiency diseases may be investigated and possibly cured on a commercial scale. Further experiments will therefore be undertaken to test these possibilities.

The above results are published at this stage in the hope that they may be of use to other workers along similar lines.

SUMMARY.

1. The possibility is discussed of diagnosing deficiency diseases by injection experiments in the actual plantation and even of curing the diseases in certain cases by the same method.

2. The serious damage which sometimes results from the injection of nutrient salts has up to now made the use of this method risky.

3. This damage has been shown to vary according to:

i. the variety;
ii. the rootstock on which the tree is worked;
iii. the compound in which the element is injected;
iv. the concentration of the injected solution;
v. the total amount of substance injected.

4. Varying amounts of an injected substance caused equal damage when the volume of the solution increased as the square of the amount of substance.

5. 0·25 per cent. solutions of all the nutrient salts tested may be injected with safety. Sufficient quantities are absorbed in about twenty-four hours to bring about growth increases as great as any caused by the heaviest applications to the soil.

6. All nutrient elements may be injected without ill effects if suitable compounds are selected and if they are dissolved in sufficient water.

7. Two growth increases resulting from the injection of nutrient salts are mentioned.

ACKNOWLEDGMENT.

The one author is grateful to the Director and Staff of the East Malling Research Station for the facilities afforded him during his visit.

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INJURY TO APPLE TREES DUE TO PARAFFIN OIL USED FOR THE CONTROL OF WOOLLY APHIS

By **ROBERT McKAY, A.R.C.Sc.I.**

Department of Plant Pathology, Albert Agricultural College, Glasnevin, Dublin

In October 1931 portions of some diseased maiden apple trees originating from a nursery in the south of Ireland were forwarded for examination by the Department of Agriculture. The trees had been broken off close to the graft, but it was evident that a canker had been present in this region, although no fungal parasite was found. Further specimens were received from the same source in January 1932, and it was then stated that the disease was spreading and had appeared on trees which were healthy in October. As the trouble appeared to be a serious one, the material was at this stage handed over to the author for investigation by Professor P. A. Murphy.

A description of the injury is as follows: As in the case of the first lot of specimens, all the trees were maidens, and the canker was for the most part confined to the callus at the junction of stock and scion. The injury varied from an open canker half an inch in diameter exposing the wood to a slight darkening of the bark, and in the latter case it was found on cutting that the injury was more extensive than the external appearance indicated. The tissues of bark and cortex above and below the cankers were sound. The trees were of the varieties Beauty of Bath and Newton Wonder.

Further diseased specimens of the same two varieties were supplied on request in March 1932, some of which showed an incipient discoloration at the graft, and some appeared normal. On planting, it was found that the former died without the buds of the scion showing any movement, although the stock afterwards sprouted; while the latter developed during May a canker which either girdled the tree and killed it, or failing this, ceased development and usually became callused completely over the same season. The appearance of the cankers in May and June was characteristic. Beginning about the end of April, the bark just above the union of stock and scion became brown, and subsequently sunken and cracked, leading to an open canker. The dead area was surrounded by a black line outside of which was a light green border about 1 mm. wide, which simulated the zone of invasion of a parasite but was really due to the rapid formation of a new callus around the wound (Figs. 1 and 2).

Reports from the Department and from the nursery indicated that the trouble was still serious, being confined chiefly to maiden apple trees, especially of the variety Beauty of Bath, which was found very susceptible, and also Newton Wonder. All attempts, however, to discover a parasite or to reproduce the disease experimentally met with complete failure. The most usual organisms isolated were bacteria, and occasionally fungi, but when inoculated into Beauty of Bath, Newton Wonder and other varieties of apples, both during the growing and dormant season, all the cultures proved non-pathogenic.

In the Winter of 1932-3 a further report from the nursery was received through Mr. Dearnaley, Horticultural Inspector, indicating that the trouble had almost disappeared, for only four cankered trees could be found. These were again sent us for examination and were found to show open cankers situated higher up on the trees than in the previous lots. Furthermore, traces of woolly aphis were found on the cankers, and since both laboratory work and the field reports had by now indicated that the disease was not of parasitic origin, it was surmised that it might have arisen from some treatment used for the control of woolly aphis. This was abundantly proved in subsequent work, as will now be shown.

EXPERIMENTS ON THE USE OF PARAFFIN OIL.

Treatment for woolly aphis under nursery conditions is usually carried out with methylated spirit where only a few trees are affected, but where the outbreak is severe the use of spirit is uneconomical and paraffin oil is used. The first treatment was therefore carried out with this liquid.

In December 1932, a number of maiden apple trees of the varieties Beauty of Bath and Newton Wonder were painted with paraffin oil at the junction of scion and stock. There was no immediate result and the occurrence of injury did not become apparent until the following April, when it was seen that the buds on the treated trees were not swelling, and the bark of the painted portions was found on cutting to be dead, although no evidence of this was visible externally. From the middle of April, however, the bark over the treated parts became visibly discoloured, and the trees then (or ultimately) presented a similar appearance to that of the specimens received from the nursery. None of these trees survived, all dying as a result of being girdled at the point of treatment, though the stocks below sprouted and produced normal shoots (Fig. 4).

Owing to this result a series of treatments with paraffin oil was carried out on apple trees of different ages each month from May to October 1933. A total of thirty trees was included in the experiment, made up of the following varieties:

Allington Pippin.
American Mother.
Barnock Beauty.
Beauty of Bath.
Bramley's Seedling.
Charles Ross.
Cox's Orange Pippin.
Cox's Pomona.
Gladstone.
Grenadier.

James Grieve.
King of the Pippins.
Lane's Prince Albert.
Lord Grosvenor.
Newton Wonder.
Reinette de Canada.
Ribston Pippin.
Worcester Pearmain.

The paraffin was in all cases applied with a paint brush on two or three inches of the stem or branch, but it was not necessarily brushed in. The total quantity of paraffin used throughout the whole period was somewhat less than half a pint, so it is clear excessive amounts were not applied. As a result, 80 per cent. of the treated trees showed injury, the latter varying with the variety and the age of the branch treated. The damage was most severe and showed up most rapidly on one or two year old wood, and the injury developed more quickly after oil applied in May or June than in treatments carried out in August or September. Injury, however, was not confined to the young wood, for in some cases a single application of paraffin was sufficient to kill a branch five years old. The most remarkable feature was the length of time which elapsed in the majority of cases between the time of application and the development of visible injury. The shortest period elapsing between treatment and the appearance of symptoms was eleven days in the case of treatments carried out in May, whilst the longest period occurred in trees treated late in the Autumn, when in some cases nothing was visible until growth began in the following Spring. During the Summer of 1933 fifty days frequently passed before injury could be observed, and in one instance ninety-two days elapsed before the killing of tissues due to paraffin could be confirmed.

TYPES OF INJURY DUE TO PARAFFIN OIL IN THE ABSENCE OF WOOLLY APHIS.

During the growing season if paraffin touches leaf-bearing spurs the leaves rapidly wilt and remain hanging, and the spurs soon die also. Dormant buds on treated branches are killed, and small cankers as a rule develop around them, which may heal up the same season, or in the case of susceptible varieties may encircle the shoot or branch. After treatment with paraffin on current year's shoots, the leaves wilt, and the bark becomes discoloured and rises up in flakes all over the dead portion. The killing of these shoots is often first noticed at the base, and the two year old wood below may show more or less similar effects (Fig. 3, compare Fig. 7).

On three to five year old wood, injury is first observed around the base of any dormant buds, small spurs or shoots which may be present, with the result that these are killed and a canker develops at their base. Such cankered areas are small, usually with an average diameter of half an inch. The appearance of these dead spurs or shoots is exactly similar to that produced by Blossom Wilt (*Sclerotinia cinerea* Schroet.) and might easily be mistaken for it. On branches of from three to five years of age, where no buds, spurs or shoots are present, the injury is of quite a different type, depending somewhat on the variety. In some varieties such as Bramley's Seedling and Cox's Orange Pippin nothing may be visible for sixty days after treatment, when a number of fine cracks appear in the bark. These gradually enlarge and finally develop into splits varying in length from one to four inches and up to one quarter of an inch wide. This type of injury used to be quite common on apple trees which were reguarly sprayed with caustic winter washes, and amongst practical gardeners the affected limbs were generally referred to as being " hide-bound ". In other varieties, such as Lane's Prince Albert, there is marked discoloration and the bark scales up in flakes. Microscopical examination shows that there is considerable killing of the tissues in this case, sections through the darkened areas showing the cells to be brown and dead up to a depth of about 1 mm. The first of these two types of injury is perhaps not very serious except in so far as it may allow spores of *Nectria galligena* and other parasites to gain entrance in the early stages of its development. The second type is much more serious, because the bark may be completely killed down to the wood, and generally over quite a considerable area (Figs. 6 and 7).

In the case of the two classes of injury just described, it was noticed repeatedly that the worst damage did not always develop immediately at the point where paraffin was applied, but at a little distance below it on the branch, and especially at a bend if one was present. In the case of horizontal branches the injury practically always developed, and was more severe, on the lower side of the branch. When paraffin oil is applied to an apple tree, it spreads rapidly, or as Lefroy (1) has termed it, " creeps ". In a number of five year old Allington Pippins treated at a height of about six inches on the main stems, the worst injury developed just at ground level (compare Fig. 3).

Open cankers on branches treated with paraffin oil are due to the killing of the bark over limited areas, the dead tissues shrinking, cracking and eventually falling away as growth proceeds. Such wounds may develop during the same season in which the treatment is carried out, or if paraffin is applied during September or October, the cankers may not appear until growth begins in the following Spring. Shrinkage of the bark due to treatment with paraffin oil is also of common occurrence, especially on the main stems of young trees, where the bark becomes constricted over the treated area.

INJURY TO TREES WHERE WOOLLY APHIS WAS PRESENT.

A severe infestation of woolly aphis occurred on a number of espalier apple trees in the Autumn of 1933 and a number of experiments were carried out on them. A minimum quantity of paraffin was applied in the ordinary way with a brush to the spots were the insects were feeding. As is usual, the pest was most severe on the current year's shoots and the two year old wood, and most of the treatments were carried out on these growths, though some older wood was also treated. In all cases where the insects were present and paraffin was applied severe injury followed. The harmful effects generally appeared first at the base of the shoots and on the spurs below, the bark darkening and rising up in scales and forming small cankers. In the variety Reinette de Canada the leaves wilted and remained hanging on the treated shoots, and an examination of the latter showed them to be dead. If galls due to the insect were present on the shoots, cankers developed rapidly from them, and the galls split and the bark covering them turned brown and curled back. This splitting was quite different from that which commonly results from the woolly aphis attack itself, for the latter usually occurs in Spring when growth is active, and as a rule there is little browning or killing of the adjoining tissues. It was obvious that the insect punctures facilitated the entrance of the oil, because on corresponding shoots free from woolly aphis which were treated at the same time, the injury did not develop so quickly as where the insects were present. Furthermore, a single dressing was not sufficient to eradicate the pest on the shoots and spurs, as living specimens were found on treated shoots a few days after the oil was applied. These may possibly have come in from neighbouring untreated controls on the same trees, but from their position and general appearance it was concluded that they had probably survived the treatment, as the oil was not scrubbed into the crevices (see Fig. 8).

TREATMENT WITH PETROL AND METHYLATED SPIRIT.

In September when discussing this subject with Professor Murphy, he suggested treating some trees with petrol to see the effect. It was known from parallel experiments with methylated spirits that no damage of any kind was produced, and it was thought that owing to its rapid evaporation petrol might not be so injurious as ordinary paraffin. A similar inquiry as to the possible effect of petrol was made by Mr. G. O. Sherrard, Lecturer in Horticulture, about the same time.

Injury from petrol was first observed fifty days after it was applied, but it was not visible on all the treated shoots at this time. It developed rapidly, however, afterwards on the varieties Bramley's Seedling and Lady Sudley, cankers appearing at the base of the treated shoots. In Reinette de Canada the shoots died and the leaves remained hanging on them, as was the case with paraffin oil on this variety. Woolly aphis was present on these trees but others free from the pest were also treated with petrol and they also developed cankers around the base of young treated shoots, although on older branches free from aphis no injury of any kind could be detected fifty days after the petrol was applied.

As already mentioned a number of experiments were carried out with methylated spirit. Trees free from woolly aphis, and also some where colonies of the pest were feeding, were treated with spirit. No injury of any kind ever appeared on these trees, and a second and in some cases a third heavy application of spirit was made to the same part without producing any harmful effect. Methylated spirit, therefore, can be used with complete safety for the control of woolly aphis, its high cost, however, prohibits its employment for this purpose except where only a few trees require treatment.

DISCUSSION.

Paraffin oil has been used from time to time either in the form of an emulsion or alone for the control of various insects on apple trees. Its use for the control of woolly aphis is mentioned in a number of text books and leaflets and has generally received the sanction of scientific workers. As recently as 1932 undiluted paraffin was authoritatively recommended for the control of woolly aphis on old trees.

Theobald (2) records the treatment of 2,000 trees by hand scrubbing the trunks with strong paraffin emulsion for the control of mussel scale and states: "Of the 2,000 trees treated, six were said to be killed, and ten badly damaged. These were all around the tanks where the wash was prepared, and it is probable that the first few pailfuls of wash were not properly mixed, and then the trees had an extra dose of paraffin. This may or may not be so, but according to the men working in the plantation the death was mainly due to old and repeated canker." He goes on to say: "The effect on the bark of some of the trees was very marked, peculiar irregular cracks being formed, but none of these trees have been damaged and have blossomed well." Evidently the cracking of the bark referred to was of a similar type to that described for branches of Bramley's Seedling in the present experiments.

A little further on Theobald writes: "Two trees were sprayed with pure paraffin in Winter, in neither case was any damage done to the tree, and all the scale was destroyed." Now the present writer used paraffin oil for the control of woolly aphis in a nursery in 1908-9, and no ill effects were ever observed. The outbreaks of the pest, however, were on a small scale, and it it quite possible that injury followed the use of paraffin and was overlooked, or owing to the length of time elapsing between the time of application and the appearance of injury, the two were not connected. On the other hand, the brands of paraffin oil on the market at the present time probably differ considerably from those used in 1908-9. In the experiments referred to by Theobald paraffin (Tea Rose) was used, that employed in the present investigations was sold under the name of "Royal Daylight."

After the present paper was first drafted my attention was drawn to an apparently identical type of injury to shoots of Cox's Orange Pippin recorded by Wormald (4) in 1928. The following is Wormald's conclusion in this case: "All the cankers smelt strongly of paraffin and this appears to be a case of injury caused by the application of paraffin to cut surfaces; the treatment was presumably for checking attacks by American Blight, but the true facts of the case could not be ascertained." This conclusion was evidently correct except that a cut surface is not usually essential before the injury takes place.

Theobald was also well aware of the injurious effects of paraffin even when used as an emulsion, and he says with regard to paraffin emulsions: "Except in bad attacks of scale insects they cannot be recommended for summer use, nor paraffin in any form." Emulsions were not tried in the present investigations. In America, where paraffin emulsions are largely used in Summer for the control of various orchard insects, it has been shown that injury to apple foliage often follows from their use. The more important of these publications are included in the bibliography attached to the recent article by Young and Morris (5) to which the reader is referred. In this paper the authors record observations over a period of seven years with twenty-five different kinds of oil and describe the injury produced by emulsions on flowers, fruit, buds and twigs.

Regarding the use of petrol, Theobald (3) states : "Immersion of young stock was recommended by the late Mr. Spencer Pickering, who recommended a bath of petrol and found that this treatment killed all the Woolly Aphid, and resulted in no injury to the trees, where other oils proved fatal." Judging from the injury following the use of petrol on the young wood in Autumn of 1933 such treatment could not be advised and neither paraffin oil nor petrol undiluted or unmixed should be used on apple trees. It should be remembered that the most severe infestation of woolly aphis is nearly always on the young wood and this is the part most easily killed by paraffin or petrol, irrespective of whether the insect is present or not.

Throughout the literature in these islands dealing with woolly aphis and "canker", the association of the latter with the injuries caused by the insect is often noticed, and it is generally assumed that such cankers are due to the fungus *Nectria galligena*. In the light of the present results, the possibility cannot be excluded that they have been caused in some instances by the use of a dressing for the control of the insect pest. Where small spurs and buds are killed by paraffin, early stages of the resulting injury closely resemble that caused by the canker fungus in its incipient form and the damage might very easily be mistaken for canker in the absence of microscopical examination.

Finally, winter washes derived from mineral oils are being extensively used at the present time, and are usually considered quite safe as dormant sprays. Nevertheless, the author has met a number of growers who maintained that injury follows the use of some of these washes. A sample of such injury occurred in the orchard at Glasnevin in the Spring of 1933, which was brought under my notice by Mr. Sherrard just at the time when results were being obtained from paraffin oil, and it seems worth while to describe it. In May when the buds were bursting it was seen that many of the buds on the young shoots, especially of Worcester Pearmain, were going " blind ", the trees presenting a very curious appearance. At the cut end of the young shoots the top two or three buds were developing weak leaves whilst none were appearing on the buds below, giving the shoots a tufted appearance at the top. On close examination, practically all the buds on the basal portion were found to be dead and when touched with the finger they fell off. Whilst the injury was most severe on Worcester Pearmain it was not confined to this variety, as investigation showed other similar dead buds scattered throughout other varieties both on young shoots and two year old wood. No cankers resulted, and early in the Summer weak subsidiary buds developed leaves where the original buds had been killed, and the trees then appeared more or less normal. Whilst there were no controls in this case, the injury could not be attributed to any other factor except the winter spray, and the appearance of the dead buds was exactly like that produced by paraffin oil before the canker stage appears.

ACKNOWLEDGMENTS.

The writer wishes to express his thanks to Professor P. A. Murphy for diseased specimens originally received, and for assistance and encouragement throughout the investigation. Thanks are also tendered to Mr. J. Dearnaley, Horticultural Inspector, for reports on the nursery where the injury first appeared and for other information ; to Mr. J. Carroll, Agricultural Zoology Department, and Mr. G. O. Sherrard, University Lecturer in Horticulture, each of whom placed apple trees at the author's disposal for experiments.

SUMMARY.

1. A canker which resulted in the death of maiden apple trees in a nursery was traced to the use of mineral oil for the control of woolly aphis.

2. It was proved experimentally that paraffin oil injured or killed the shoots of a number of apple varieties, the most susceptible being Beauty of Bath and Newton Wonder. Wood of the current or previous year was most severely injured, but branches up to five years old were killed by a single treatment.

3. Various types of injury produced by paraffin oil on apple trees are described. The injury was aggravated by the presence of woolly aphis.

PLATE I.

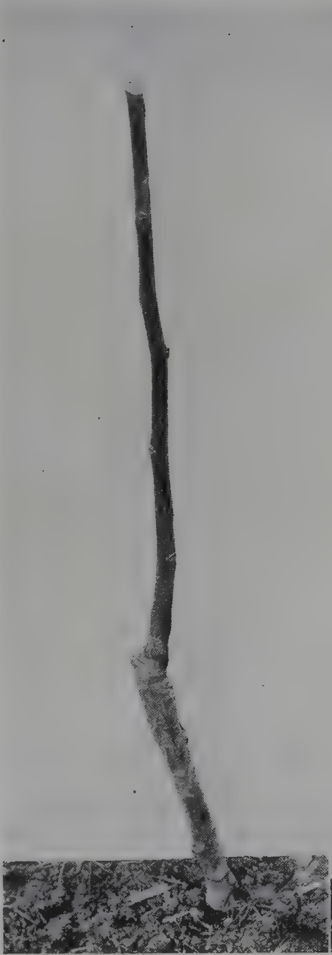

FIG. 1.

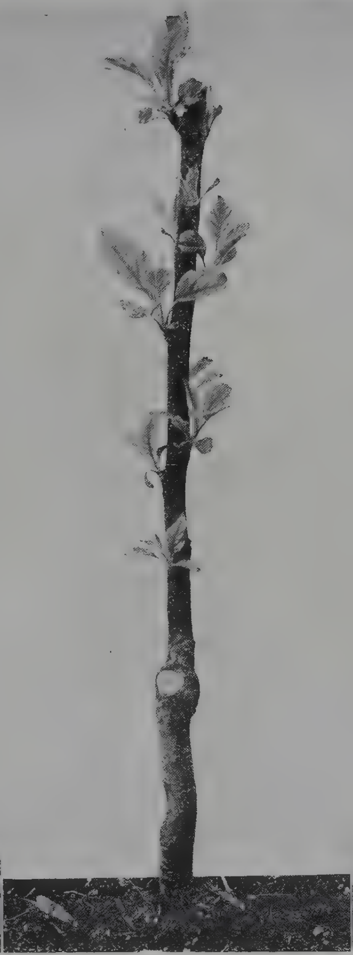

FIG. 2.

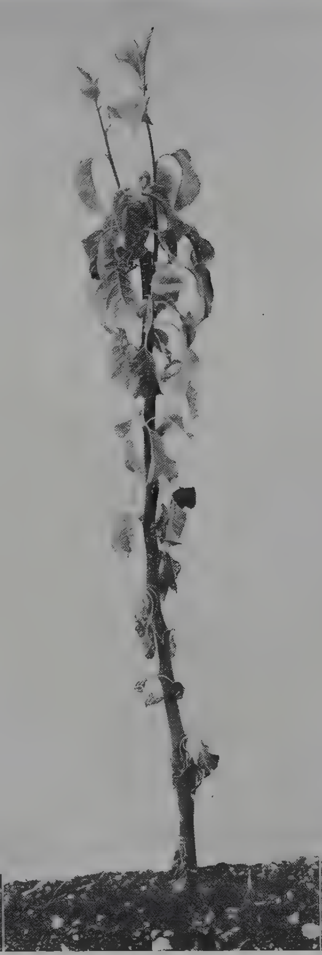

FIG. 3.

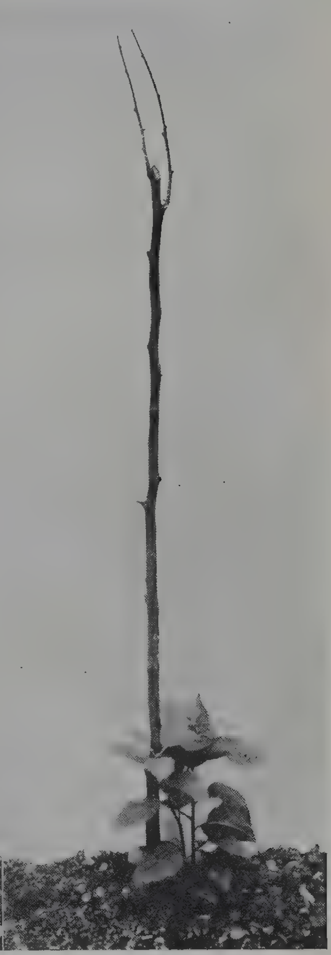

FIG. 4.

PLATE II.

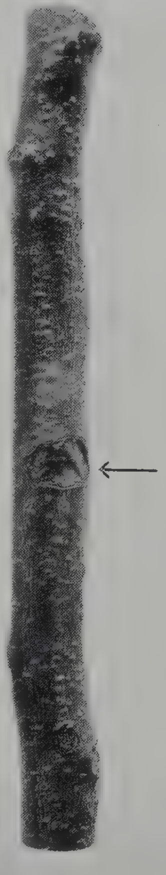

Fig. 5.

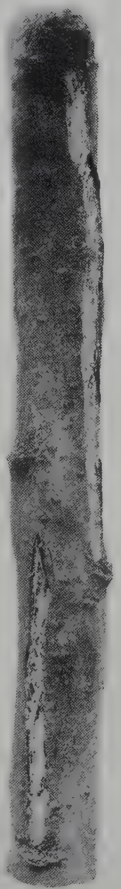

Fig. 6.

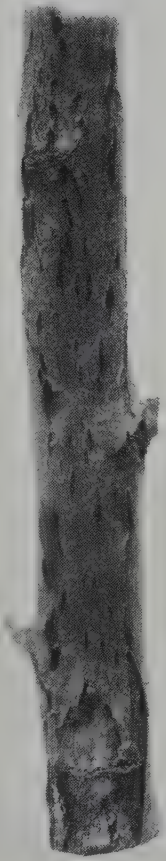

Fig. 7.

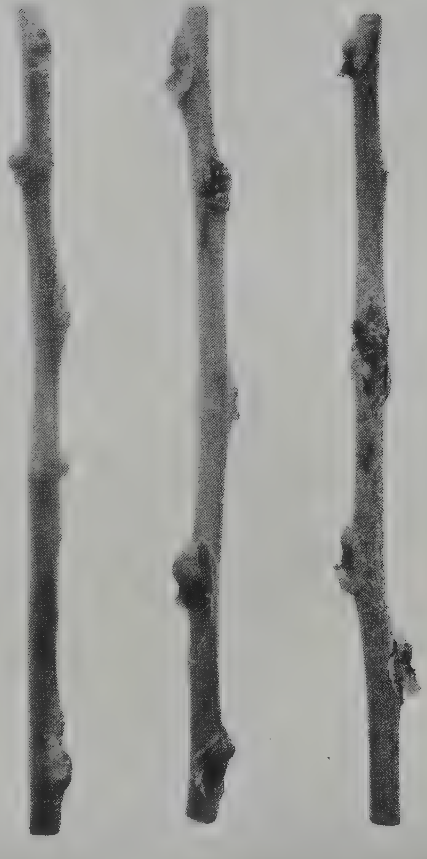

Fig. 8.

4. Paraffin oil or petrol should not be used alone on apple trees either in the dormant season or in Summer, but applications of methylated spirit were found to be harmless to the tree.

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EXPLANATION OF FIGURES.

Fig. 1.

Young apple tree, Beauty of Bath, received from Nursery in March 1932, and immediately planted. At time of planting there was a slight browning of the bark at callus which later developed into a canker and killed the scion. Photograph taken in May 1932, when tree was completely dead. This is typical of maiden trees treated with paraffin oil in Autumn and Winter.

Fig. 2.

Similar to Fig. 1 and of same variety, but no discoloration was visible at planting time. A partial canker developed above the callus in May but it did not encircle the stem completely, with the result that the tree recovered and the canker healed over.

Fig. 3.

Beauty of Bath, one year old tree, painted with paraffin oil in middle of the stem in May 1933, photograph taken twenty-eight days after treatment. Note leaves wilting on spurs over the treated portion.

Fig. 4.

Same tree as in Fig. 3, showing complete death of upper portion. Growth below is from stock. Photograph taken in August 1933.

Fig. 5.

Middle of main stem of year old Newton Wonder treated with paraffin in May at the same time as tree in Fig. 3. Note cankers which developed around the buds (see arrow). Photograph taken in November 1933, when cankers had partially healed over.

Fig. 6.

Five year old branch of Bramley's Seedling showing characteristic cracks on lower side following treatment with paraffin oil. Treatment carried out at end of June 1933, photograph taken in October same year.

Fig. 7.

Lower side of six year old horizontal branch of Lane's Prince Albert showing killing and scaling-up of bark which is dead down to the wood following treatment with paraffin oil. Paraffin applied in end of June; photograph taken in October same year.

Fig. 8.

Three shoots showing galls due to woolly aphis, two treated with paraffin oil showing the cankers which developed at right, one control at left. Photograph taken in November 1933, fifty-six days after treatment.

THE SEASONAL CYCLES OF NITROGENOUS AND CARBOHYDRATE MATERIALS IN FRUIT TREES

I. THE SEASONAL CYCLES OF TOTAL NITROGEN AND OF SOLUBLE NITROGEN COMPOUNDS IN THE WOOD, BARK AND LEAVES PORTIONS OF TERMINAL SHOOTS OF APPLE TREES UNDER TWO CULTURAL SYSTEMS—GRASS PLUS ANNUAL SPRING NITRATE AND ARABLE WITHOUT NITROGENOUS FERTILIZER

By D. V. KARMARKAR, M.Sc., Ph.D.*
Long Ashton Research Station

INTRODUCTION.

THE numerous investigations relating to the nutrition of fruit trees which have been carried out during the past half century have shown very clearly the fundamental importance of nitrogen supply to fruit trees in practical fruit growing. Results demonstrating this fact have been obtained in connection with all the major fruit growing areas of the world, and hence it is not surprising that the attention of scientific horticulturists should have been closely focussed on problems of nitrogen metabolism.

In the older experiments on orchard manuring carried out in the U.S.A., the pre-eminent feature of the results was the effect of nitrogen applied to trees growing under sod systems of culture. It was shown that such trees are often apparently severely nitrogen starved and large responses both in vegetative growth and cropping followed from the application of dressings of nitrogenous fertilizers such as nitrate of soda and sulphate of ammonia. In such cases, the addition of nitrogen not only affected the above two factors, but also such features as foliage size and colour, time of defoliation, colour of the bark and size, colour and keeping qualities of the fruit were all altered. When similar manurial treatments were applied to trees—especially apples—growing under cultivated conditions, the responses to the dressings were often very small and frequently negligible, and from such observations it became apparent that the so-called grass effect, studied by Pickering (30) and attributed by him to the toxic action of the growing grass could be explained, at least in part, on the basis of nitrogen deficiency. Thus the relationships of cultural practices to nitrogen supply became evident.

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* This paper embodies the results of investigations by Dr. Karmarkar, which were presented by him for the Degree of Doctor of Philosophy in the University of Bristol, June 1933. Dr. Karmarkar proceeded to India before the determinations on the leaves were completed and the estimations of the nitrogen fractions in these for August, September and October have been made subsequently by Mr. J. O. Jones, M.Sc.

The present account of the investigations has been prepared by Dr. T. Wallace, mainly from the original thesis. The section of the Discussion relating to the leaves has been added by Dr. Wallace, who has also modified and extended the original conclusions.

Later, relationships between nitrogen metabolism and practical aspects of fruit culture have been approached mainly from the starting point of the $\frac{\text{carbohydrate}}{\text{nitrogen}}$ theory of Kraus and Kraybill (15) who, from work on the tomato, formulated an hypothesis to explain the vegetative and reproductive activities of the plant in terms of the ratios and amounts of carbohydrate and nitrogenous substances within it. These findings were applied to fruit trees by such workers as Hooker (13), Harvey (10), Roberts (35), Murneek (25), Potter and Phillips (31) and others, and attempts have been made to link up the ratio with problems of vegetative growth and fruit bud formation, biennial bearing, fruit setting, time of manuring and other cultural and pruning practices.

In the older investigations along these lines, it was customary to consider nitrogen only as "total nitrogen" as determined by the ordinary Kjeldahl procedure, but as the work has progressed, it has become evident that a true picture of the part played by nitrogen in the metabolic activities of the plants can only be obtained by detailed fractionation of the total nitrogen in the various portions of the trees. The need has also been shown for studies of the cycles of the nitrogen fractions within the trees and in recent times a few investigations of this sort have been attempted.

At Long Ashton Research Station, the point has been reached in connection with nutritional studies when it seems desirable to embark on detailed chemical studies relative to nitrogen metabolism. By means of pot and field experiments, coupled with chemical determinations, the main practical effects of nitrogen deficiency on fruit trees have been established. These have been summarized previously by Wallace (49).

The connection between cultural systems and nitrogen supply has also been determined and field plots established to study the problems in detail. Special plots have also been in existence at Long Ashton since 1920 for the purpose of examining critically the effects of the time of application of manures, especially nitrogen, on tree behaviour, and these have now reached a stage suitable for prosecuting intensive investigations. In this particular case it would appear desirable to determine whether the application of quick-acting nitrogenous fertilizers affects the normal nitrogen cycle within the tree. Moreover, it appears that nitrogen studies may be profitably applied to rootstock problems, since certain features of rootstock effect have been shown by Swarbrick and Naik (40) to be related to characters suggesting $\frac{\text{carbohydrate}}{\text{nitrogen}}$ relationships.

As a preliminary to these various studies, an investigation has been made of the nitrogen cycles within the terminal shoots of two sets of apple trees on a plot at the Long Ashton Station where the effects of two cultural systems are under examination, viz. clean cultivation and grass culture plus an annual spring dressing of nitrate. In this work it has been possible to determine the cycles of certain nitrogen fractions under arable conditions without the interfering factor of the addition of a nitrogenous fertilizer, and to study the effects of grass culture and the application of a nitrogenous fertilizer at one point in the seasonal cycle.

The results have shown clearly that total nitrogen and certain fractions of this constituent, especially of the water soluble non-protein portion, exhibit definite cycles associated with certain seasonal growth phases, and that the two cultural systems affect the nitrogen conditions within the trees. Moreover, it is clear from the results that the separation of terminal shoots into the fractions leaves, bark and wood is desirable in any study of this kind.

HISTORICAL.

NITROGEN FRACTIONATION METHODS.

Knowledge regarding the many processes involved in the metabolism of nitrogenous compounds is still very limited. A successful study of such problems was impossible until the nature of these nitrogenous bodies was definitely known. Fischer's monumental work on the constitution of proteins paved the way. Hausmann (11) later developed a method for the fractionation of organic nitrogenous compounds into a few characteristic groups and his method was subsequently improved by Van Slyke (45, 46). While this latter method does not involve a complete analysis of the proteins and the intermediate products, as in Fischer's method, nitrogen compounds are grouped according to the particular grouping of the nitrogen in the amino-acids or in the protein molecule, and these groups are estimated as such. The main advantages of the Hausmann method are that it is less complicated than Fischer's original method, and that it can be carried out with relatively small amounts of material.

Using this method, Osborne, Wakeman and Leavenworth (28) in U.S.A. made an extensive study of the proteins and the water soluble constituents of the alfalfa plant and showed that much useful information regarding the character of the nitrogen compounds in this plant could be obtained in this way.

The first comprehensive investigation of nitrogen metabolism in higher plants in this country in which the Hausmann procedure was followed was carried out by Chibnall (3). He investigated the quantitative and qualitative changes occurring in the leaves of the runner bean throughout a complete growth cycle. Protein was found to undergo very little or no qualitative change, while the water soluble fraction varied considerably in composition. Decreases in the total nitrogen and protein nitrogen were observed during periods of rapid growth. Asparagine and ammonia nitrogen in the soluble fraction were very low throughout. Evidently there was a relationship between certain nitrogen fractions of the soluble part and protein nitrogen. Thus nitrate and mono-amino nitrogen varied with the total protein content, suggesting that these simpler forms of nitrogen are utilized in protein synthesis. "Other nitrogen"—the fraction found by difference—varied inversely to protein nitrogen and appeared to be a product of degradation. Chibnall suggested that the possible course of nitrogen metabolism in the runner bean begins with the assimilation of nitrates, which are converted into mono-amino acids and protein, the latter finally breaking down to "other nitrogen".

NITROGEN RELATIONSHIPS IN FRUIT TREES.

Various workers have investigated the composition of the tissues of such plants as the apple, but the value of the results is usually limited, due to the fact that, in the majority of cases, the material analysed has been collected only at one period of the year. On the other hand, analyses of materials representative of growth cycles have been relatively few and this is especially true in the case of fruit trees. This latter type of work is of special value in comparative studies since variations in chemical composition corresponding to differences in physiological states of plants may be more evident at particular seasons of the year, i.e. at particular stages in the seasonal development of the plant, while at other periods, differences may assume minimum values.

Leclerc du Sablon (16) analysed the carbohydrate, nitrogen, ether extract and moisture content of various trees including the pear and chestnut. Richter (34) studied the nitrogen and ash content of leaves of different fruit trees at different stages of growth.

Butler, Smith and Curry (1) were the first to make a detailed study of the distribution of food materials in the shoots, trunk and roots of apple trees at different periods of the seasonal cycle. This investigation showed that the amounts of nitrogen present in the roots and branches were always greater than in the trunk. During the dormant period, the one year old branches contained relatively the most nitrogen. Between bud break and the blooming period of the tree, the loss in nitrogen became very marked in the one, two and three year old branches. From the cessation of active growth to leaf fall, all the branches and roots became relatively richer in nitrogen. These authors concluded that the nitrogenous reserve materials were stored mainly in the younger branches whence they passed to the actively growing regions at the end of the dormant season.

Hildreth (12), working upon the hardiness of apple trees, studied the nitrogenous constituents of the apple and found that the nitrogen reserve in the shoots was fairly low and the amino-nitrogen content extremely small. The most notable point about the nitrogen content was its consistency throughout the greater part of the year. The greatest fluctuation occurred in spring when there was a sudden rise about the time of bud swelling. He observed that the percentage of organic nitrogen was appreciably higher in the hardy Duchess variety than in the Jonathan. The amino-nitrogen was most abundant during periods of vegetative activity.

A very comprehensive investigation of the regional and seasonal distribution of moisture, carbohydrates, nitrogen and ash in two to three year portions of apple twigs has been carried out by Traub (44). He divided the twigs into four different parts: (1) periderm, (2) cortex-phloem, (3) outer xylem, (4) inner xylem-pith. It was found that the nitrogen peak was reached in March-April just preceding rapid growth extension. After a marked decline in nitrogen with rapid shoot growth, the content of the element was relatively constant during the summer. The nitrogen content also remained practically constant throughout the winter after leaf fall. The amino nitrogen was highest during the growing season, decreasing as the rest period approached and reached a minimum during the dormant season. The protein nitrogen, on the other hand, was highest during the dormant season afterwards decreasing and reaching a minimum in June.

Sullivan and Kraybill (38) have studied the seasonal changes in the composition of Stayman apple trees with reference to the forms of nitrogen, but full details of the work are not yet available. It was noted, however, that above ground the soluble nitrogen was higher in the bark than in the corresponding wood tissues, while in the roots the soluble nitrogen was higher in the wood than the bark. Between November and December there was an increase in both total and all other forms of this element. From April to July, there was a continued decrease in total nitrogen on the dry weight basis.

Sullivan and Cullinan (39) have recently examined the carbohydrate and nitrogen relationships in apple shoots as influenced by soil management. Their results indicated that the terminal shoots of trees receiving tillage with cover crop showed a greater content of total nitrogen, expressed as percentage of dry weight during the growing season, than trees in sod or alfalfa. The sod trees had the lowest total nitrogen content in spite of the fact that they were the only trees receiving an organic nitrogen fertilizer. The differences in total nitrogen between the various plots were much greater during the active growing season than during the dormant season.

Thomas (42) was the first to undertake a rather complete nitrogen fractionation of woody tissues such as apple twigs. He analysed entire apple twigs without separating them into bark and wood. Like Chibnall, he found that the composition of the protein of the leaves was the same throughout the life period, and he concluded therefrom that although there was no absolute proof that a single protein was present in the leaves, his results were strongly suggestive of such a condition. He found nitrate nitrogen in the aerial parts only at one period, viz. in the buds just as they were opening. He considered that in this species nitrates are transformed into amino-acids for the most part in fine roots. The soluble protein was found to be very small throughout the cycle. In this work the importance of the possible rôle of the " rest " nitrogen compounds was also pointed out. His results concerning this fraction appeared to confirm Chibnall's view that it is connected with protein degradation.

A similar study of the nitrogen metabolism of the pear was carried out by Mulay (23). He chose as his material for the study, current year's shoots. Unlike Thomas, he separated the bark from the wood. He found that new growth commenced with high total nitrogen in the bark which decreased as the growth season advanced. After the slowing down of growth, the total nitrogen increased, reached a maximum in winter, and fell again when new buds began to open, drawing upon the reserves in the shoots. Insoluble nitrogen appeared to be mainly affected in these seasonal changes. The total nitrogen in the wood showed changes of a somewhat similar character to those in the bark. Changes in soluble nitrogen were mainly responsible for the seasonal fluctuations in wood but this did not apply to the bark. Soluble proteins, forming only a small fraction of the total nitrogen, both in the bark and in the wood, showed an increase in late summer and autumn, and a fall in late winter and spring.

As there were significant changes in the amount of non-protein nitrogen in the shoots during their seasonal growth, Mulay considered that a further study of this non-protein nitrogen fraction as regards quality might be very helpful. He therefore carried out further fractionation of the non-protein nitrogen (24). The data showed that new growth started with high concentration of amide and amino nitrogen in both bark and wood. Both these forms decreased in amount as the growth progressed, reaching a minimum in autumn and winter, and increasing again when new buds began to swell. Amide nitrogen in wood was much higher than the amino nitrogen in the period of active growth. Basic nitrogen, which was at its lowest in June, reached its maximum some time before the start of new growth and then fell rapidly. On hydrolysis of the non-protein nitrogen, humin nitrogen in the bark and rest nitrogen in the wood decreased considerably, causing corresponding increases in the amide, amino and basic nitrogen fractions.

It will be seen from the foregoing literature that, although very few studies of the cycles of the nitrogen fractions in fruit trees have been carried out, it is apparent that investigations of this sort are likely to throw considerable light on the processes of nitrogen metabolism within the plants and on the effects of nutritional factors on these processes.

EXPERIMENTAL.

MATERIALS.

The trees from which the materials used in the present investigations were obtained are growing on an experimental plot at the Long Ashton Research Station. The plot is utilized for the purpose of studying the effects of two cultural systems on apple trees—clean cultivation without any nitrogenous manuring, and grass culture plus an annual spring dressing of nitrate, either nitrate of soda or nitro-chalk at 5 cwts. per acre per annum being given. The plot, which contains twenty varieties of bush apples on Malling No. II root-stock, set at 15 feet square, was planted in the autumn of 1923 and, until the autumn of 1928, a system of clean cultivation was followed, intercropping with raspberries being practised until the winter of 1926-27. At the end of the period, the plot was divided into four strips, running east to west across the plot at right angles to the rows of the individual varieties, and alternate strips were sown down to a pure culture of perennial rye grass. Since that time the grass has been kept short on the grass strips by periodic cuttings each season between mid-May and September, the cuttings being left *in situ*.

Since planting, annual dressings of sulphate of potash and of phosphatic manures have been applied and, from the spring of 1929, the differential nitrate treatment has been given to the grass strips according to plan.

The variety Newton Wonder was selected for the present work. On both arable and grass plots the trees of this variety have made good growth, although it is evident that the vigour of those in the arable areas is now stronger than that of the trees on the grass strips.

During the period of sampling—March 1932 to March 1933—the trees on the arable sections would be described as vigorous whilst those on the grassed areas were of medium vigour and showing some effects of the grass treatment by a slightly pale colour of the foliage and of the barks. During the season of the investigation the crops on all trees were negligible.

In the early years of the experiment the trees had received spur pruning treatment, but from 1931 no pruning was done so as to eliminate possible nutritional responses due to this factor.

FIELD SAMPLING.

Eight trees were available under each treatment from which to obtain shoots for analytical purposes. Sampling was carried out at monthly intervals, always under fair weather conditions, when leaves and shoots were free from external moisture, and an effort was always made to collect the samples for each month on consecutive days in the order—grass, arable. As will be seen from the tables, on no occasion was there any appreciable interval between the collection of the comparable samples. In order to avoid diurnal fluctuations (42) in the composition of the shoots, sampling was always carried out between 10.30 a.m. and 11.30 a.m. (9.30 a.m. to 10.30 a.m. during "summer time").

The shoots selected for analysis on each occasion were current season's growths, usually leader shoots or side shoots which had developed in exposed positions and which were fairly comparable in age and growth characters with the leaders. Each sample was a composite one from all the available trees, consisting of 50-60 shoots. Each shoot taken comprised the whole length of the current season's growth.

The number selected per sample appeared adequate in view of Mulay's (22) results in his statistical study with pear shoots, from which 35 to 40 shoots were recommended to give a fairly representative sample. On the occasion of the last sampling, some difficulty was experienced in obtaining the requisite number of suitable shoots, especially on the grass plot, but apart from this it was felt that the samples were representative of the type of shoots required for the work.

Immediately on cutting, each sample was wrapped in waterproof paper and conveyed to the laboratory where it was prepared for analysis without delay.

Laboratory Sampling.

On arrival at the laboratory, the shoots were first wiped clean with a dry cloth. The total weight of the sample was ascertained and then the lengths of individual shoots measured. When the shoots were devoid of leaves, they were divided into the portions bark and wood but, during the active growing season, the division was into laminae of leaves, bark plus petioles and wood. In connection with the leaf samples, since these were generally too large to handle conveniently, it was necessary to take aliquots and this was done by selecting representative leaves along the shoots, by which means the age factor was eliminated.

However desirable it may be to work with fresh material, this is not always possible in studies of the present kind and some method of preservation is necessary. Any method followed should combine convenience with negligible effect on the constituents to be analysed. Usually there are three possible methods of preservation: (1) freezing, (2) preservation in alcohol, and (3) desiccation. Of the above three methods drying seems to be usually preferred. The range of temperature employed has been a point widely discussed and varies a great deal with different investigators. Chibnall (4) has shown the inaccuracy of low temperature drying. Thomas (41) in his studies of the influence of temperature on the nitrogenous constituents of apple shoots considered 60° C. as the optimum temperature of desiccation. Link and Tottingham (19) have shown that if tissues are dried at 95° C. in a well aerated chamber for a short period to destroy enzymes and then to constant weight at 60°-65° C., the changes in carbohydrates are extremely small. Pearsall and Wright (29) found the above method of drying very satisfactory for preserving material for analysis for a study of the nitrogenous constituents. Changes in the nitrogen distribution due to proteolytic activity during the time of desiccation at 55° C. were shown to be negligible by Lincoln and Mulay (18). These authors have further shown that drying at 50° C., though quite satisfactory for bark and wood, apparently caused hydrolysis of proteins in leaves as evidenced by the increase in the non-protein nitrogen fraction. They showed that drying at high temperatures reduced the amount of protein extracted and appeared to give a truer picture of the non-protein nitrogen in all the leaves, bark and wood samples.

In view of the above results, high temperature drying was preferred in the present investigation, since it was concerned mainly with the non-protein nitrogen.

The samples, divided into bark, wood and leaves, as described above, were kept in an oven at 95° C. for fifteen minutes to kill any enzymes present and then finally dried to constant weight in an oven at 70° C. When dry, the samples were reduced to powder in a Christy and Norris Laboratory Mill, the final grinding being done by hand so that the material could easily pass through a 60 mesh sieve. As the material has a tendency to absorb moisture, the samples were further dried after grinding and subsequently preserved in well-stoppered glass bottles until the time of analysis.

DISCUSSION OF CHEMICAL METHODS.

The estimations carried out consisted of the following: (1) total dry matter, (2) total nitrogen, (3) total water soluble material, (4) total non-protein nitrogen, (5) nitrate, (6) ammonia, (7) acid amide, (8) humin, (9) basic, (10) imide and mono-amino, (11) rest, and (12) proteose.

Since the methods adopted by various workers in investigations of the present type have been very varied and the several determinations are usually open to criticism, it will be appropriate before stating the details of procedure adopted to discuss the reasons for the final selections of the methods used.

Non-Protein Nitrogen. In the study of non-protein nitrogen the first and most important step in the procedure is the satisfactory extraction of the fraction. Thomas (42) agreed with Tottingham, Schulz and Lepkovsky (43) who had found that direct extraction with water was satisfactory for investigations involving the partition of nitrogenous constituents as compared with Chibnall's (2) procedure employing ether as a cytolytic agent. Jodidi (14) used boiling water for extraction of the non-protein nitrogen in his investigations on the nitrogen metabolism of spinach. This procedure is objectionable not only because a certain degree of hydrolysis of proteins may take place, but many nucleins are decomposed by boiling water with formation of hypoxanthine etc. (42). Thomas employed water at 40° C. for extraction. Mulay preferred extraction with cold water to extraction with warm water as it is less removed from the temperature conditions prevailing in the living tree.

In the present investigations, distilled water freed from ammonia by long boiling and then cooled to room temperature was employed.

Having obtained the extract, the next step necessary is the satisfactory separation of soluble proteins from the non-protein nitrogen fraction. Acetic acid is a very common reagent employed for this purpose. Pearsall (29) used Stutzer's reagent. Thomas (41) found that colloidal ferric hydroxide had many advantages over the reagents in general use for the separation of proteins from their hydrolytic products.

The water soluble protein fraction in apple shoots is naturally very low and moreover drying the samples at higher temperatures, as has been already observed, reduces the amount of protein extracted. Acetic acid was, therefore, used to coagulate any soluble proteins obtained in the extract. No appreciable quantity of coagulum was obtained in any case during the investigations.

It has been found that, in the case of aqueous extracts, the modified Kjeldahl-Gunning-Arnold method to include nitrates (32) does not give accurate values for total nitrogen in the presence of nitrate nitrogen. Mulay, who was unable to demonstrate the presence of nitrates in his material, employed the simple Gunning method. Thomas (42) also used the Kjeldahl method without modification for nitrates. Harvey (10), however, has found appreciable amounts of nitrate nitrogen in spurs. As there was always the possibility of the presence of nitrate nitrogen, an accurate method, applicable in the presence of nitrate nitrogen, was sought. Two possible methods are the "Devarda" alloy method and the reduced iron-sulphuric acid method, although the latter has been criticized because of the acid nature of the reducing medium. In tests of the accuracy of these two methods, it was found that both gave accurate results in the presence of nitrate nitrogen and the Devarda method was finally selected for its easy working. The procedure adopted embraced the modifications of Phillips, as recommended by Loomis (20) for plant materials.

Ammonia. The determination of ammonia, present in the form of ammonium salts, was carried out by vacuum distillation of the extract using MgO as alkali. Chibnall estimated ammonia by distilling at ordinary pressure with MgO. Certain nitrogenous compounds like proteoses, however, are decomposed at 100° C. even by such a mild alkali, ammonia being liberated, and the latter procedure is thus unsuitable. Thomas used the method described by Van-Slyke (45) which is in all essentials the same as that described by Longi (21) who distilled at a temperature of 38°-40° C. with MgO. He noted that neither urea nor asparagine were decomposed under these conditions. Aeration methods for the determination of ammonia are quite accurate and have been successfully employed. A low temperature distillation method, however, was preferred as it is more convenient and equally accurate as the more tedious aeration procedure.

Nitrate Nitrogen. A number of methods have been suggested for the determination of nitrate nitrogen in plant materials, but of these not one has withstood the criticisms of several investigators in this field. Sessions and Shive (36) in putting forward the Devarda method for nitrate nitrogen, as modified by them, have discussed briefly the three main methods used for the determination of this constituent and the several modifications suggested. The three chief methods are (1) the reduced iron method, (2) the Kjeldahl-Gunning-Arnold method, (3) the Devarda method.

The Kjeldahl-Gunning-Arnold method gives satisfactory values if water free material is employed. In the case of extracts, therefore, it is necessary to evaporate the extract to dryness. Ranker (33), discussing the inaccuracies of the Devarda method, showed that though successful elsewhere, it did not give real nitrate figures in the case of plant materials and suggested that if any significant correlation should be found between the nitrogen fraction as found by Devarda method and the physiological activity of plants, the term "Devarda" nitrogen instead of the usual term nitrate nitrogen should be employed.

There are two main objections against the reduced iron method: (1) the acid reducing agent may have a hydrolysing influence on the amide nitrogen; (2) in the presence of an acid reducing agent, the nitrate may be reduced to the intermediate stage of nitrite, the acid liberating nitrous acid from which it may react in turn with amino groups which may be present, either in the form of amino acids or proteins, with the liberation of nitrogen.

When the procedure of the reduced iron method was so modified that the heat was not applied too rapidly, the loss of gaseous nitrogen apparently did not occur, though, as Nightingale (26) has pointed out, the possibility of loss of nitrogen due to improper manipulation constitutes a serious objection to the method as well as the necessity of working with amide-free solutions. The Ulsch method in the form advocated by Vickey and Pucher (47) has been employed in the study of tobacco leaves undergoing curing operations (48). It was found in this investigation that if the reaction mixture is not warmed, satisfactory results can be obtained, the duplicates always agreeing within 0·1 c.c. of N/50 alkali. From the data obtained it is evident that even when the amide nitrogen fraction reaches its maximum and is present in large amounts, no trace of hydrolysis due to the acid nature of the reducing agent occurs.

Organic Nitrogen Fractions. Thomas (42) and Mulay (24), both use two methods for fractionation of the organic nitrogen. These methods differ from each other with respect to the vigour of hydrolysis of the original complex. In one method, 4 per cent. hydrochloric acid is employed while, in the other, acid of 20 per cent. strength is used. The results obtained by the two methods adopted by Thomas differed from each other with respect to (a) the distribution of the mono-amino and the di-amino acids and (b) the substances determined as amide nitrogen. Mild hydrolysis with 4 per cent. HCl gives as amide only asparagine and the closely related glutamine, which are the most important acid amides found in plants. Bearing in mind the importance of asparagine nitrogen in such plant physiological studies, the mild hydrolysis was used in the present instance. For the general fractionation, the method of Hausmann (11) as modified by Osborne and Harris (27) was essentially followed.

Acid Amide. On hydrolysis of the extract with 4 per cent. hydrochloric acid, nitrogen present in the form of amide is transformed into ammonia and the acid amide nitrogen can thus be estimated in the latter form.

Humin. During the hydrolysis an insoluble black product generally known as humin is obtained. This is supposed to be formed by the interaction of mono-amino, di-amino acids and carbohydrates. It is usually separated with the MgO used for the distillation of ammonia and the nitrogen content of the residue determined.

Basic. Nitrogen compounds of a basic nature, mainly di-amino acids, are precipitated by 20 per cent. phosphotungstic acid and the amount of nitrogen in the precipitate is a measure of the quantities of such basic nitrogen compounds.

Imide. The total nitrogen contained in the filtrate from phosphotungstic acid consists of the nitrogen representing mono-amino acids and the nitrogen of heterocyclic compounds such as proline and oxyproline. The latter has been designated as "Imide" nitrogen.

Mono-amino-acid Nitrogen. Sorenson's (37) formol titration method was employed.

By the procedure adopted it has been found possible to account for all of the non-protein nitrogen. Thus it was found by analysis that the fraction designated as rest nitrogen is absorbed by the precipitate formed on the addition of barium hydroxide employed for removing the phosphotungstic and sulphuric acid mixture from the filtrate after the separation of basic nitrogen by the latter reagents.

Proteose was determined by the salting out by zinc sulphate in acid medium—this being the method employed by Nightingale (26) and Chibnall (3), though the latter preferred ammonium sulphate to zinc sulphate in his later investigations (4).

DESCRIPTION OF CHEMICAL METHODS USED.

These are given in detail in the Appendix.

RESULTS.

In order to facilitate interpretation of the analytical results on the nitrogen fractions, details regarding sample numbers, dates of collection, condition of the shoots when gathered, and certain other relevant data concerning the wood, bark and leaves portions of the shoots are given in Tables I and II.

The results of the analyses of the nitrogen fractions by the foregoing methods, together with data relating to water soluble material in the shoots, are presented graphically in Figures 1-33.

In connection with the results for nitrogen, the values obtained for mono-amino acid N have been included under the heading imide N, since the amounts found were comparatively small. Nitrate N in each set of graphs has been placed at the end of the series. This has been done in order to emphasize the fact that the values are those obtained by the procedure followed and require critical confirmation as "true" nitrate N.

As regards the general method of expressing the amounts of the various nitrogen fractions, the following points should be noted in the figures: total nitrogen is expressed as mgms. per 100 gms. dry matter; protein N and non-protein N are also shown on this basis and as percentages of total N; all fractions of the non-protein N are given as mgms. per 100 gms. dry matter and also as percentages of non-protein N. This method allows of following the cycles of the fractions of the non-protein N as actual amounts and as percentages of this constituent.

In Tables I and II it will be seen that all analytical data are calculated on a dry matter basis.

DISCUSSION.

In order to obtain a clear understanding of the rôles of the various constituents requiring discussion, it is advisable to divide the year covered by the investigation into periods corresponding to well defined physiological states of the trees. Such a division should take into account the most important stages of the seasonal growth cycle, such as bud swelling, period of shoot elongation, autumn defoliation and dormancy. The following division has been adopted for the present purpose.

1. *Period of Bud Swelling*. This period covered approximately the months of April and May. At the commencement of the period the fruit buds were showing the first visible signs of seasonal activity and by the end of May full blossoming had been reached whilst terminal and lateral shoot buds had

TABLE I.

Arable Plot—Data Relating to Wood, Bark and Leaves Samples.

Sample No. and date of Sampling.	Condition of Shoots.	Mean length of Shoots. (cms.)	Wood and Bark Portions only of Shoots.			Dry Matter in Fresh Wood. %	Dry Matter in Fresh Bark. %	Dry Matter in Fresh Leaves. %	Dry Leaves in Total Dry Shoots (Wood + Bark + Leaves). %
			Total Dry Matter in Total Fresh Weight. %	Dry Wood in Dry Shoots. %	Dry Bark in Dry Shoots. %				
1932. 2 March 17	Dormant	40·1	47·2	47·6	52·4	56·0	44·3		
4 April 19	Practically dormant. Terminal buds woolly shape. Just moving	41·9	45·5	48·2	51·8	55·8	42·0		
6* May 25	Practically full blossom stage. Shoot buds broken to small leaves. No shoot growth	37·4	38·4	50·3	49·7	47·9	27·2		
8† June 23	First new shoots, green and succulent and with fully grown leaves	18·4	29·4	24·6	75·4	29·3	32·4	36·2	72·3
10 July 23	No terminal buds yet. Shoots hard except tip 1½″ to 2″	36·3	34·6	39·2	60·8	39·4	34·7	37·5	62·1
12 Aug. 30	Terminal buds formed. Shoots hard to tips. No secondary growth	45·4	39·1	47·0	53·0	46·3	38·5	39·1	54·5
14 Sept. 26	As August 30th	46·2	41·8	44·6	55·4	51·3	40·5	39·5	51·5
16 Nov. 1	Foliage showed very slight tinting but no appreciable defoliation of terminal shoots	49·4	46·4	47·6	52·4	55·2	44·5	39·5	43·6
18 Nov. 29	Practically defoliated. A few leaves remaining at tips	50·2	46·5	50·9	49·1	54·6	45·2	36·0‡	4·2‡
1933. 20 Jan. 3	Dormant	48·7	45·2	52·9	47·1	54·1	44·0		
22* Feb. 2	Dormant	46·0	46·6	51·9	48·1	54·6	45·3		
24* Mar. 1	Dormant. Difficulty in obtaining shoots ..	41·8	46·2	48·1	51·9	55·1	46·3		

* Shoots scarce these dates. † First new season's growths. ‡ Practically defoliated.

TABLE II.

Grass Plot—Data Relating to Wood, Bark and Leaves Samples.

Sample No. and date of Sampling.	Condition of Shoots.	Mean length of Shoots. (cms.)	Wood and Bark Portions only of Shoots.			Dry Matter in Fresh Wood.	Dry Matter in Fresh Bark.	Dry Matter in Fresh Leaves.	Dry Leaves in Total Dry Shoots (Wood + Bark + Leaves).
			Total Dry Matter in Total Fresh Weight. %	Dry Wood in Dry Shoots. %	Dry Bark in Dry Shoots. %	%	%	%	%
1932. 1 Mar. 16	Dormant	38·6	50·0	45·1	54·9	57·9	48·6		
3 April 18	Practically dormant. Terminal buds woolly stage. Just beginning to move	43·2	46·6	48·2	51·8	56·9	43·1		
5* May 23	Practically full blossom stage. Shoot buds broken to small leaves. Shoot growth negligible	40·5	39·0	51·8	48·2	48·1	34·4		
7† June 22	First new shoots, green and succulent with fully grown leaves	18·5	29·1	25·9	74·1	29·9	30·9	36·2	74·0
9 July 22	No terminal buds formed. Shoots hard except 1½″ to 2″ at tips	36·4	34·2	39·6	60·4	39·4	33·4	35·6	61·3
11 Aug. 29	Terminal buds formed. Shoots hard to tips. No secondary growth	48·1	39·9	49·1	50·9	46·8	38·2	34·8	53·4
13 Sept. 21	Practically as August 29th	44·7	42·0	45·0	55·0	52·0	41·0	38·6	51·9
15 Oct. 31	Foliage very slight tinting being slight defoliation of old wood, but no appreciable defoliation of terminal shoots	43·3	47·3	44·9	55·1	58·3	46·3	39·0	45·4
17 Nov. 28	Practically defoliated. Few tinted leaves remain near tips of shoots	46·1	46·8	48·8	51·2	56·2	46·4	36·9‡	4·0‡
1933, 19 Jan. 2	Dormant	45·5	46·6	48·8	51·2	54·9	45·4		
21* Jan. 31	Dormant	43·6	46·2	49·5	50·5	53·2	45·6		
23* Feb. 28	Dormant. Great difficulty in obtaining sample	41·2	46·9	49·6	50·4	55·5	47·5		

* Shoots scarce these dates. † First new season's growths. ‡ Practically defoliated.

broken and the shoots were mostly clothed in small whorls of leaves. No shoot elongation, however, occurred during the period.

2. *Period of Shoot Elongation.* This period, which covers the time of active shoot elongation, lasted for just over two months, June, July and part of August. By the end of the period, the new shoots had ceased to increase in length, the tips had become hard and terminal buds were formed.

3. *Period of Full Growth.* This period covers the time between the formation of terminal buds and the appearance of autumn tints, and occupied late August, September and most of October.

4. *Period of Leaf Fall.* In late October the leaves began to assume the autumn tints, turning yellowish brown. The leaf fall period comprised the latter part of October and the whole of November, defoliation being almost complete by the end of the latter month.

5. *Period of Dormancy.* This is a long period and extended over the winter months, December, January, February and March.

In the following sections, the salient features of the data relating to the various constituents are discussed, the above mentioned periods being used to indicate the various stages in the seasonal cycle.

DATA FOR WOOD AND BARK.

Water Content. Tables I and II present data for total dry matter of shoots from both arable and grass plots throughout the year and from these the cycles for water content of the shoots can be followed. No appreciable difference between the water content of shoots from the two plots can be observed. Water content shows an increase during the period of bud swelling. With the appearance of young shoots in June, it is at its maximum and then falls off steadily as growth progresses, reaching a steady low level from October onwards—the values only changing between 53 and 55 per cent.

Percentage of Wood. The proportion of wood to bark—Tables I, II—is very low in the young shoots but as the shoots become mature, the percentage of wood increases and eventually constitutes about 50 per cent. of the dry matter of the shoots. The percentages of wood in shoots from the grass and arable plots are practically the same for most of the season, but during the dormant period 1932-33 the proportion of wood on the grass plot was generally slightly lower than that on the arable.

It may be seen from the tables that the shoots obtained for the samples from the arable and grass plots were comparable as regards the lengths of the shoots on every occasion. The determinations of water content of shoots and of the percentage of wood in dry shoots also show that there was no marked difference with respect to the general growth features between the shoots from the two sets of trees.

Total Nitrogen (Figures 1, 12).

Wood (Fig. 1). The total nitrogen content of wood from mature shoots of the arable plot is consistently higher than that of wood from comparable shoots of the grass plot. It should be noted, however, that the value for the grass plot exceeded that of the arable area at the June sample when the shoots were young. This may be due to a difference in time for the peak values in the two cases.

For both sets of trees, the cycles of total nitrogen are very similar, slight irregularities being shown on the grass plot August value and on the arable plot December value.

The total nitrogen content decreases during the period of bud swelling. The young shoots in June are very rich in the constituent but the content falls rapidly as the shoots become more mature. From the end of August, when shoot elongation is complete, the total nitrogen content remains practically steady in both cases until the end of the dormant period. During this period, the total nitrogen of wood from the arable plot varies approximately between 0·60 and 0·70 per cent. of the dry wood and that from the grass plot between 0·50 and 0·55 per cent.

Bark (Fig. 12). The bark in both cases is always higher in its total nitrogen content than the wood except in June when the value for the young wood exceeds that for bark. During the cycle, the total nitrogen of bark varies between 0·75 and 1·60 per cent. of dry bark and that of wood (excluding the June sample) between 0·30 and 1·00 per cent. of dry wood approximately. The total nitrogen content of bark from arable samples is always higher than that of bark from the grass plot, the curves in the two cases being very similar in form.

The total nitrogen, which is very high in March just preceding the period of bud swelling, decreases during the months of April and May and is relatively low at the end of May during blossoming and when new growth is also just beginning. This decrease is similar to that which occurs in the wood during this period. In comparison with the very prominent peak value obtained for total nitrogen in the wood of the new growths, there is only a relatively small increase in the level of the constituent in the bark of the young shoots in June. The total nitrogen decreases during the period of shoot elongation, reaches its minimum between August and September after the shoots have ceased to elongate, and then begins to rise steadily and is high again at the beginning of the period of dormancy.

These seasonal changes in the total nitrogen of bark and of wood are very similar to those reported by Mulay (23) in his studies on pear shoots. A comparison of Figs. 1 and 12 shows that the seasonal fluctuations in the total

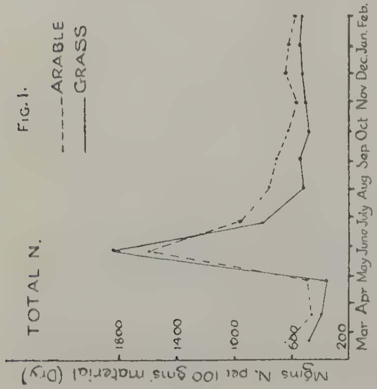


Fig. 1.
TOTAL N.
------ ARABLE
—— GRASS
Mgms N. per 100 gms material (Dry)
1800
1400
1000
600
200
Mar Apr May June July Aug Sep Oct Nov Dec Jan Feb.

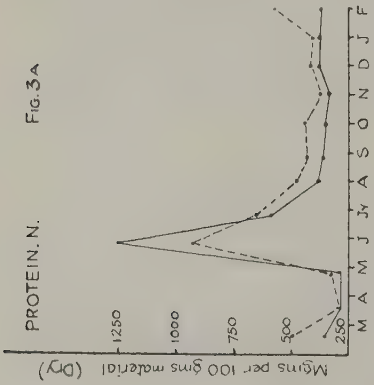


Fig. 3A
PROTEIN. N.
Mgms per 100 gms material (Dry)
1250
1000
750
500
250
M A M J Jy A S O N D J F

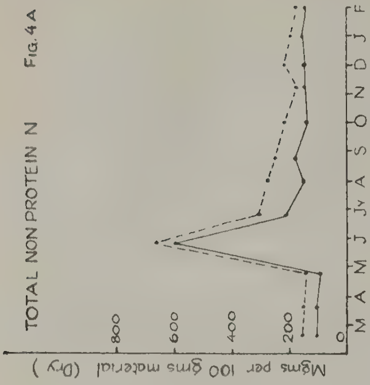


Fig. 4A
TOTAL NON PROTEIN N
Mgms per 100 gms material (Dry)
800
600
400
200
0
M A M J Jy A S O N D J F

WOOD

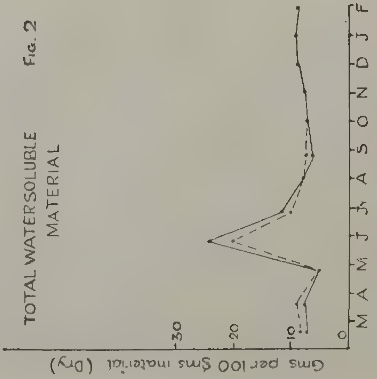


Fig. 2
TOTAL WATERSOLUBLE MATERIAL
Gms per 100 gms material (Dry)
30
20
10
0
M A M J Jy A S O N D J F

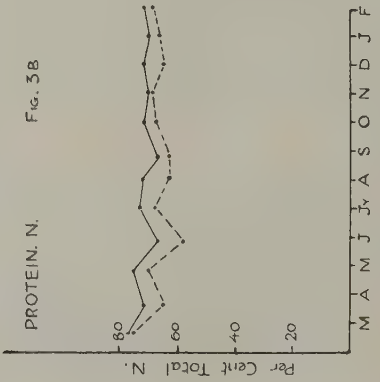


Fig. 3B
PROTEIN. N.
Per Cent Total N.
80
60
40
20
M A M J Jy A S O N D J F

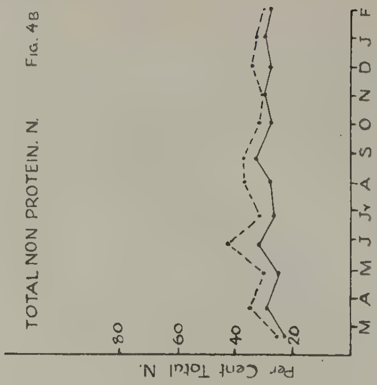


Fig. 4B
TOTAL NON PROTEIN. N.
Per Cent Total N.
80
60
40
20
M A M J Jy A S O N D J F

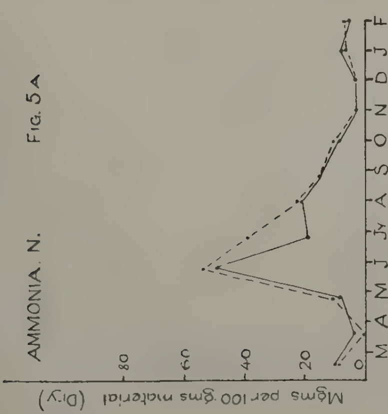


FIG. 5A

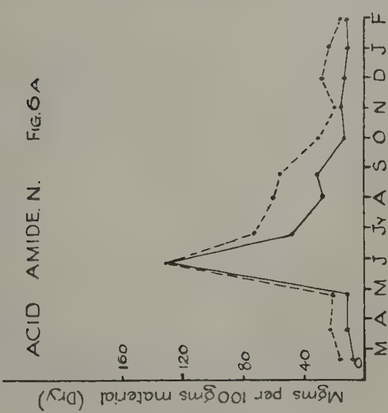


FIG. 6A

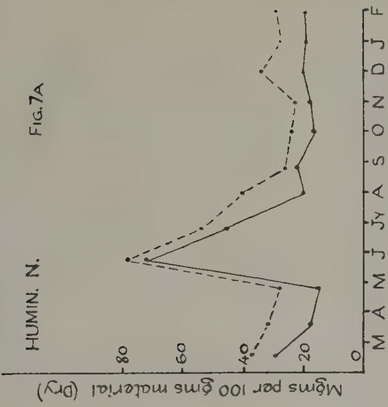


FIG. 7A

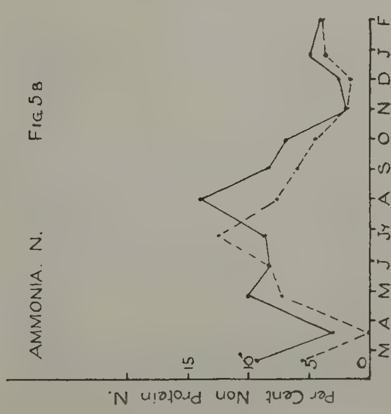


FIG. 5B

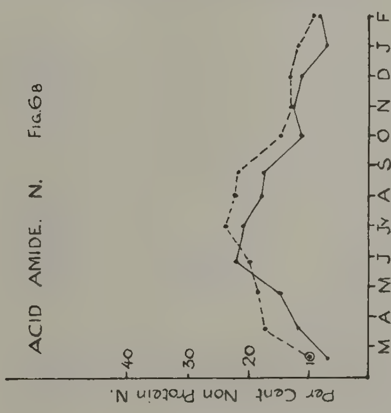


FIG. 6B

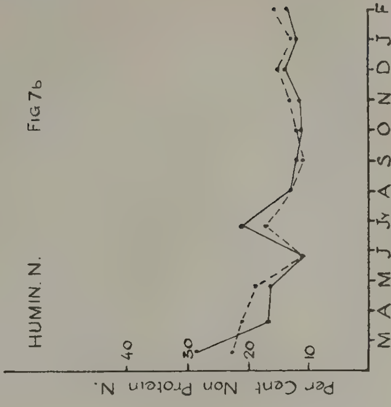
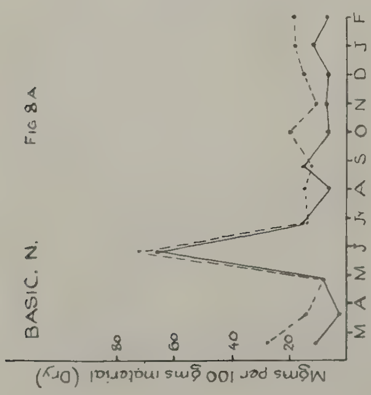


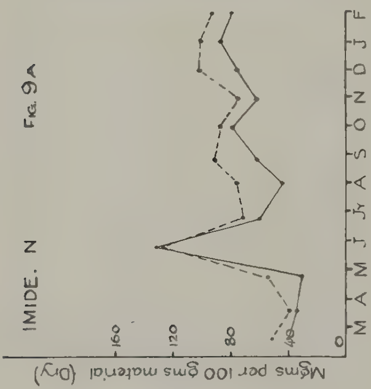
FIG. 7B

WOOD



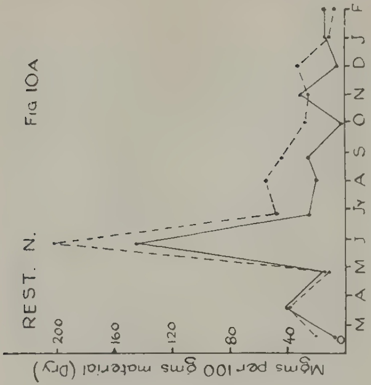
BASIC. N.

Fig 8A



IMIDE. N

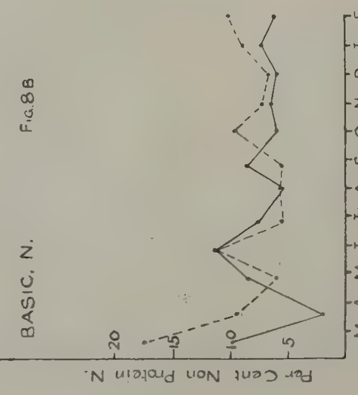
Fig. 9A



REST. N.

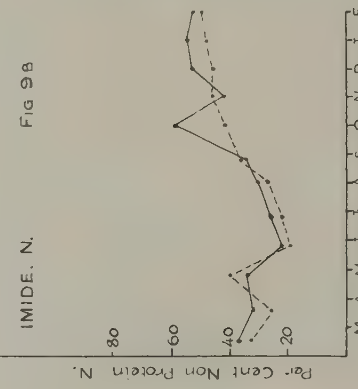
Fig 10A

WOOD



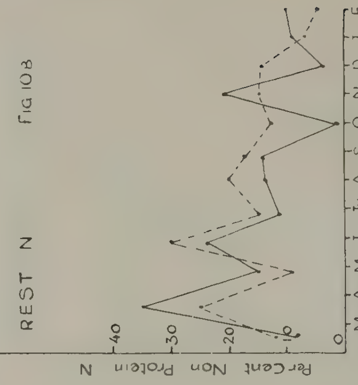
BASIC. N.

Fig. 8B



IMIDE. N.

Fig 9B



REST N

Fig 10B

WOOD

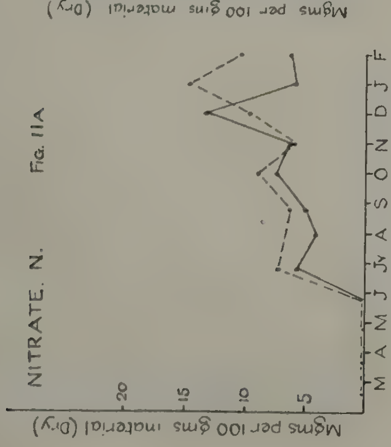


FIG. 11A

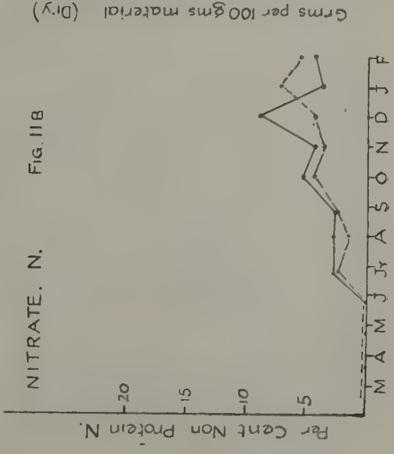


FIG. 11B

BARK

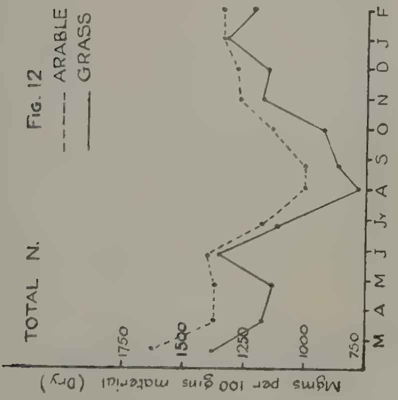


FIG. 12

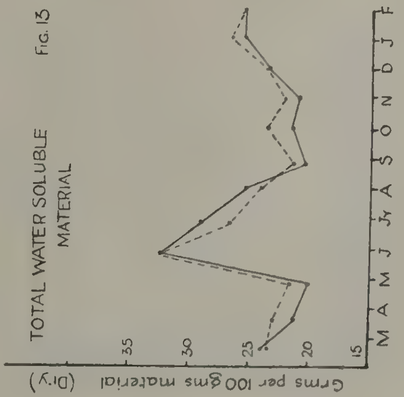


FIG. 13

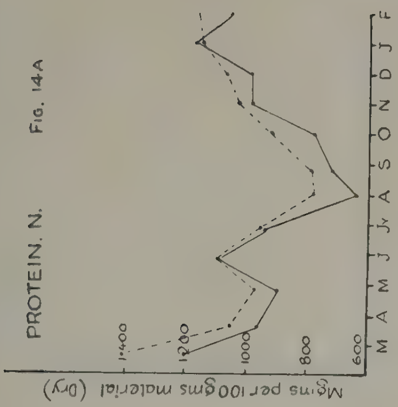


FIG. 14A

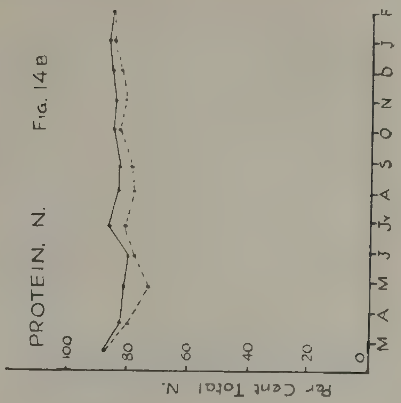


FIG. 14B

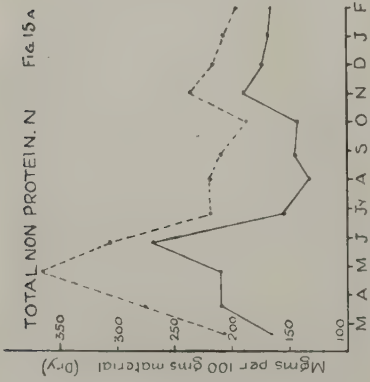

TOTAL NON PROTEIN. N

FIG. 15A

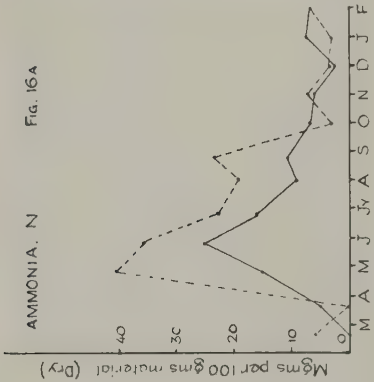

AMMONIA. N

FIG. 16A

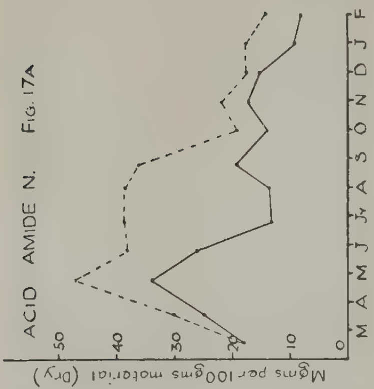

ACID AMIDE N.

FIG. 17A

BARK

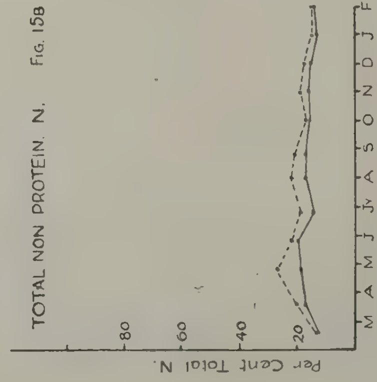

TOTAL NON PROTEIN. N.

FIG. 15B

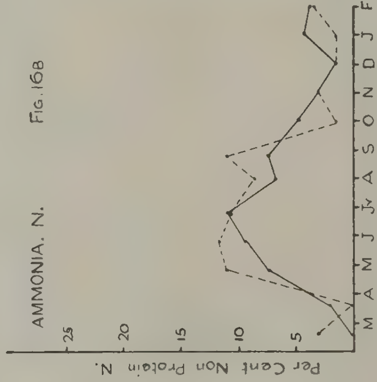

AMMONIA. N.

FIG. 16B

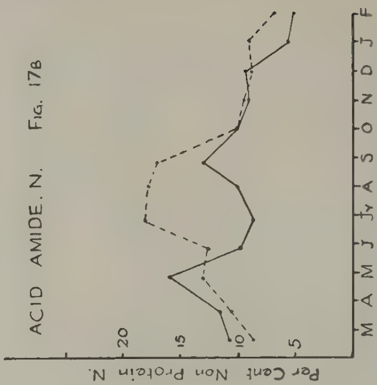
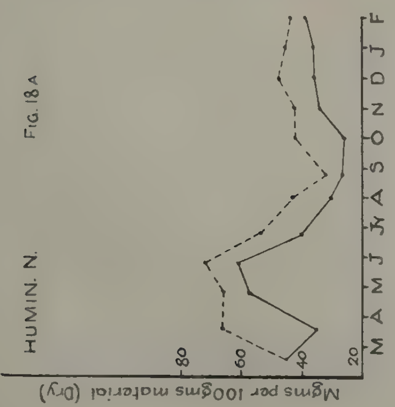
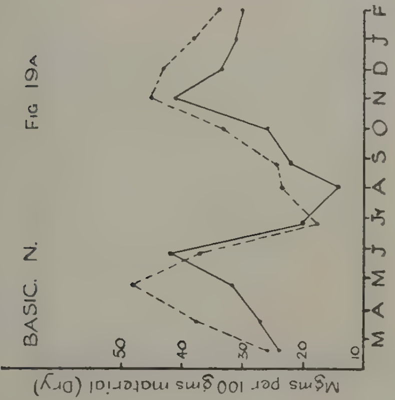

ACID AMIDE. N.

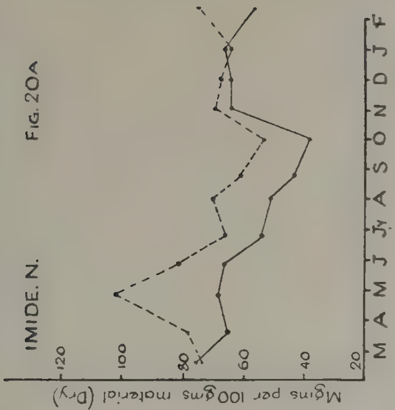
FIG. 17B



HUMIN. N. Fig. 18A

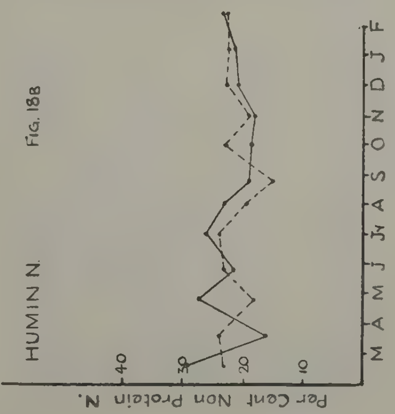


BASIC. N. Fig 19A

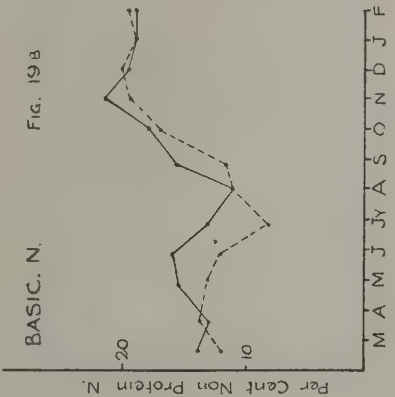


IMIDE. N. Fig. 20A

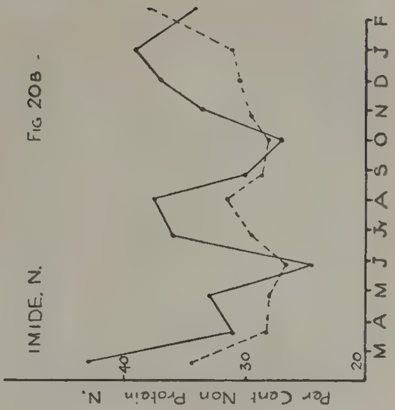
BARK



HUMIN N. Fig. 18B



BASIC. N. Fig. 19B



IMIDE. N. Fig 20B

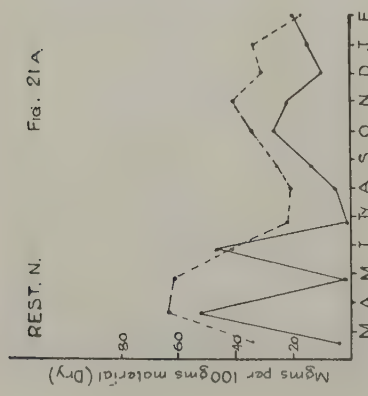


Fig. 21A.

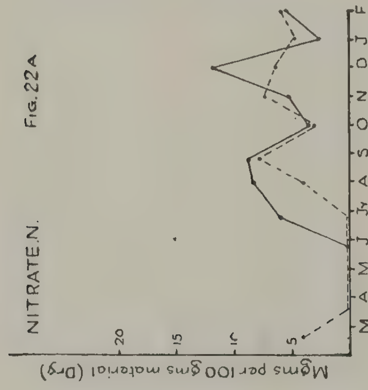


Fig. 22A

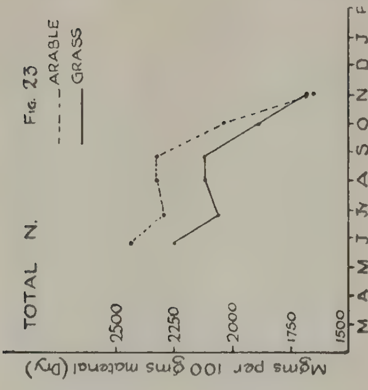


Fig. 23

BARK

LEAVES

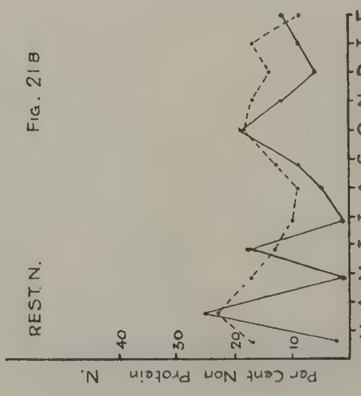


Fig. 21B

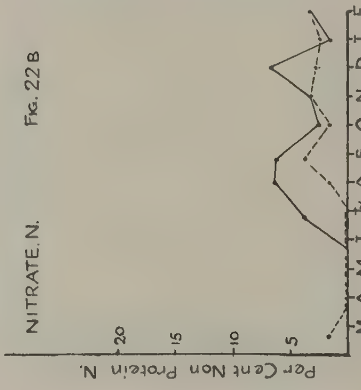


Fig. 22B

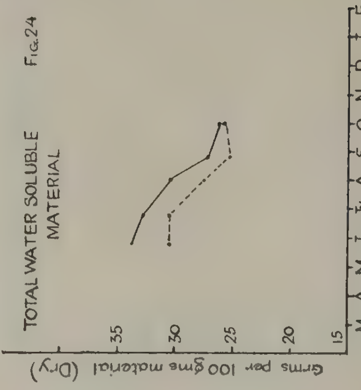


Fig. 24

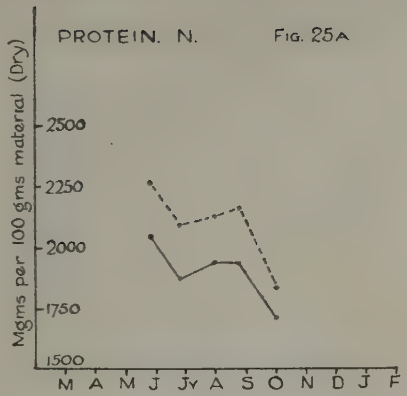


FIG. 25A

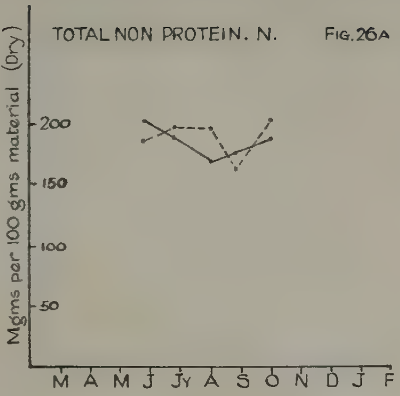


FIG. 26A

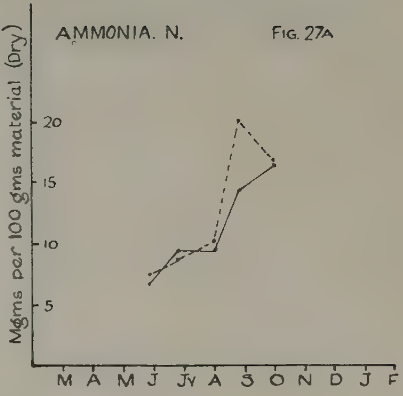


FIG. 27A

LEAVES

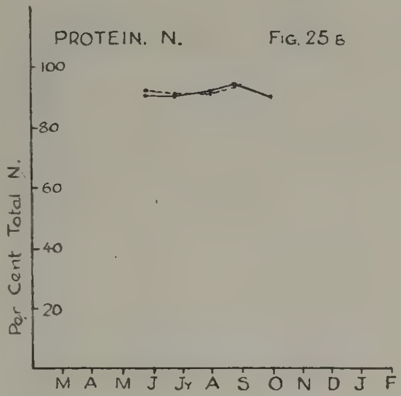


FIG. 25 B

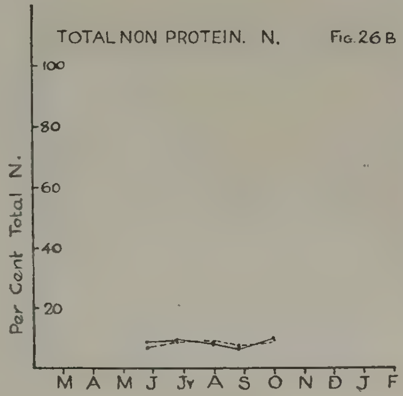


FIG. 26 B

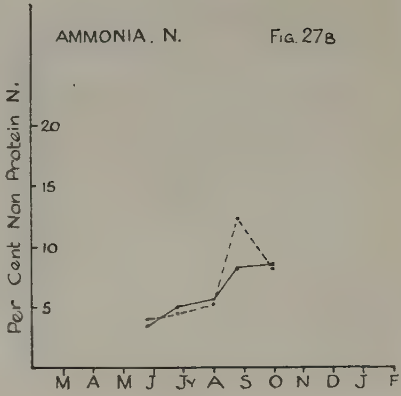


FIG. 27B

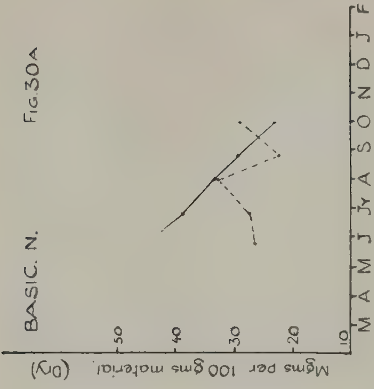


Fig. 30A

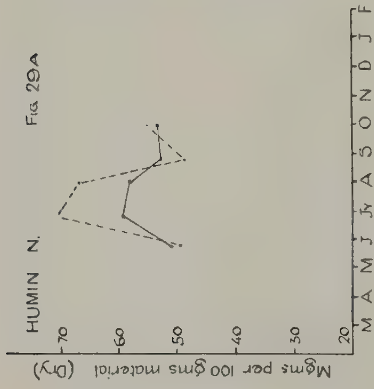


Fig. 29A

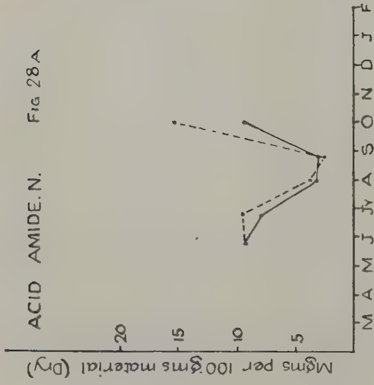


Fig. 28A

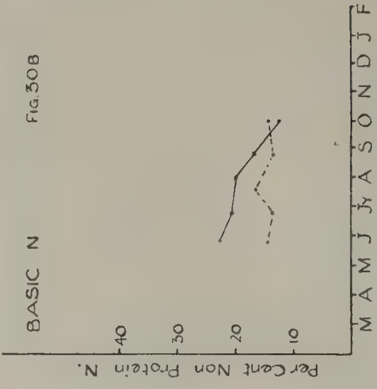


Fig. 30B

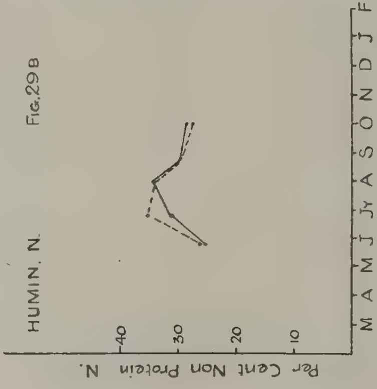


Fig. 29B

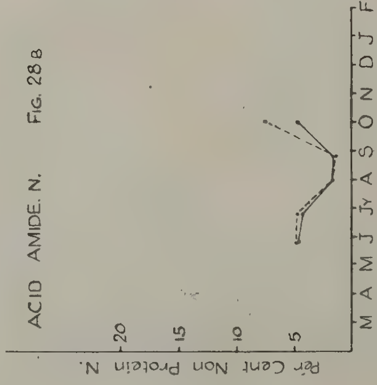


Fig. 28B

LEAVES

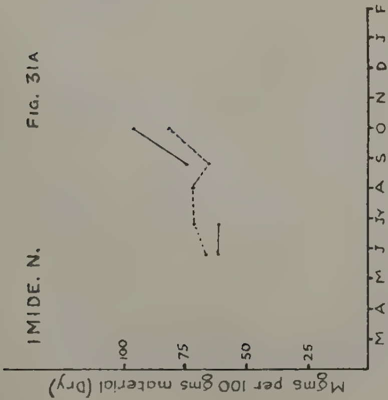


FIG. 31A

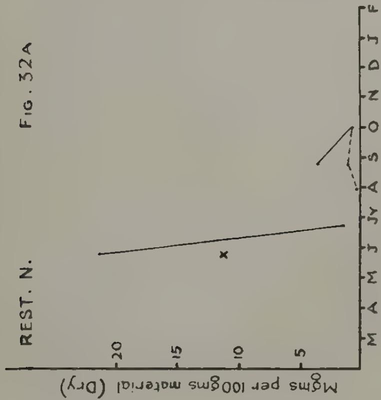


FIG. 32A

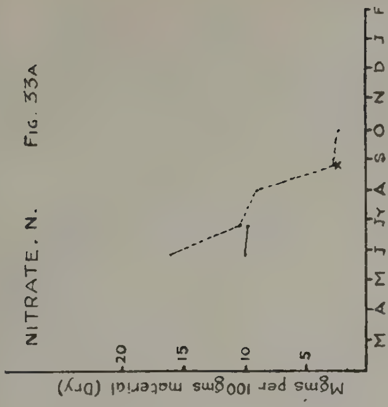


FIG. 33A

LEAVES

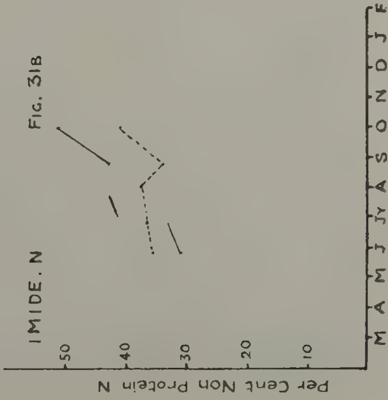


FIG. 31B

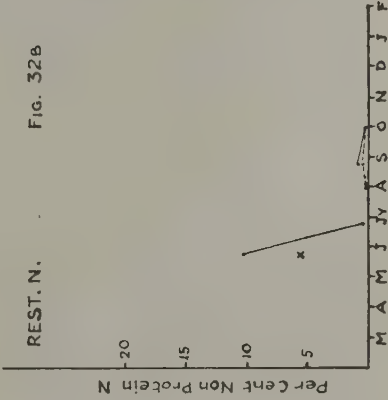


FIG. 32B

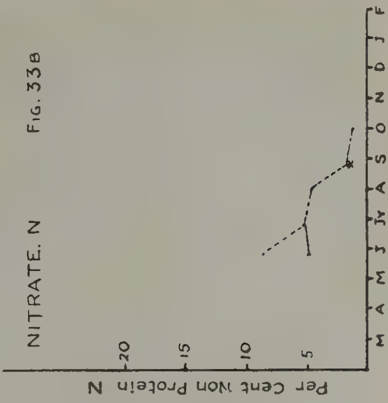


FIG. 33B

nitrogen in the wood do not appear as varied as those in the bark. This observation is also in agreement with Mulay's results on the pear.

Hooker (13) working with apple spurs, Butler, Smith and Curry (1) and Thomas (42) working with one year old apple branches find somewhat similar seasonal fluctuations in total nitrogen in their material, though none of these workers separated bark from wood.

The total nitrogen content of wood and of bark has been shown to decrease in April and May before the start of new growth. Blossoms and leaf buds develop rapidly during this period and require a high supply of nutrient materials for their development. Naturally, therefore, there must be a big draft on the nitrogenous materials in the shoots.

The very young shoots contain high proportions of nitrogen but, as active growth progresses, the content of the element falls rapidly, both in wood and in bark. Harvey (10) has observed that the tip portion of a growing shoot, which consists of meristematic tissues, is higher in its nitrogen content than the other portions of the shoot. In June, the shoots are growing actively and consist mainly of meristematic tissues and, therefore, are very rich in nitrogen. The nitrogen content falls as the shoots mature and the proportion of meristematic tissue decreases.

The higher total nitrogen content both of bark and of wood from the arable plot (excepting the stage in June) agrees with the analyses of twigs from arable and grass plots given by Wallace (49). Similar results have been reported by Sullivan and Cullinan (39) who found that the terminal shoots of trees receiving tillage showed a higher content of total nitrogen in percentage of dry weight during the growing season than those of trees in sod, in spite of the fact that only the sod trees received spring applications of an organic fertilizer.

From a study of Figs. 1 and 12, it may be observed that the spring application of nitrate fertilizer to the trees under grass seems to have no influence on the character of the total nitrogen cycle of the terminal shoot portions here considered.

Total Water Soluble Materials (Figs. 2, 13). Figs. 2 and 13 indicate the nature of the changes which occur in the amount of water soluble materials in wood and in bark respectively. The amount of such materials in wood usually varies from 6 to 9 per cent. but is higher during the period of active growth. This is approximately one third of the amount usually soluble in bark. In the latter, the amount of water soluble materials usually ranges from 20 to 25 per cent., being about 32 per cent. in the case of young shoots.

The curves both for wood and for bark of the arable and grass samples are very similar and run very close to each other. The curve for bark from the arable plot, however, is slightly higher through the leaf fall and dormant period.

Both in wood and in bark there is a decrease in the amount of total water soluble materials in May, just prior to the commencement of shoot elongation. This is similar to the decrease observed in the total nitrogen. A large amount of water soluble materials may be taken up by the developing leaves at this time, consequently leaving the shoots poorer in such materials. The percentage of water soluble material is very high in the young shoots and subsequently decreases steadily during the summer, reaches a minimum in September both in bark and in wood, and then increases gradually with the onset of the dormant period.

Protein Nitrogen (Figs. 3A, 3B, 14A, 14B). This constituent represents the difference between total N and total non-protein N. The cycles are represented by Figs. 3A, 3B, wood, and 14A, 14B, bark. It will be noted that the curves for actual amounts for both wood and bark are similar in form to those for total N. Whilst the actual amounts of the constituent are usually significantly higher in both wood and bark in the arable samples than in those from the grass plot, as in the case of total N, it will be seen from Figs. 3B, 14B, that, expressed as percentages of total N, protein invariably comprises a lower proportion in the arable samples.

Total non-Protein Nitrogen (Figs. 4A, 4B, 15A, 15B).

Wood (Figs. 4A, 4B). The cycles of the seasonal changes in the total non-protein nitrogen in wood, both from the arable plot and from the grass plot, are generally similar in character. The non-protein nitrogen content of wood from the arable plot is much higher throughout the year than that of wood from the grass plot. Omitting the June stage of young shoots, the amount of this fraction in wood from the arable plot varies from 150 to 300 mgms. per 100 gms. of dry wood, the values for wood from the grass plot ranging between 100 and 200 mgms.

The amount of non-protein nitrogen shows a slight decrease before the commencement of active growth in June. It is present in highest quantity in the young shoots. Afterwards the amount decreases, at first rapidly and then gradually, until it reaches a minimum at about leaf fall. The wood from the arable plot shows a slight increase in its content of non-protein nitrogen in December while the value for wood from the grass plot indicates a slight increase at the end of January.

Bark (Figs. 15A, 15B). The total non-protein nitrogen in the bark, as in the case of the wood, is always much higher in arable samples than in grass samples. It varies approximately between 200 and 350 mgms. per 100 gms. in the dry bark from the arable plot as compared with the range of about 150 to 250 mgms. in the bark from the grass plot.

With the exception of one stage in May, the cycles of changes in the bark, both from the arable plot and from the grass plot, are generally similar. In bark, from the arable area, the peak is reached in May while in bark from the grass plot, although an increase is shown in April, the amount of the non-protein nitrogen remains practically unchanged in May. With the advance in growth, the non-protein nitrogen falls and eventually reaches a low level in September and October. There is a sudden rise in November and then a slow decrease over the dormant period.

Both Thomas (42) and Mulay (23) have found an increase in the non-protein nitrogen during the active growing period. In the present case, however, the non-protein nitrogen shows a definite regular decrease during that period.

In April and May, when there is a slight decrease occurring in the amount of total non-protein nitrogen in wood, there is a large increase in the amount of this fraction in the bark. The bark is, at this period, a seat of great activity of the tree and is the terminus of nutrient materials conveyed for the developing buds. This fraction expressed as a percentage of the total nitrogen also shows a steep rise at this period. It appears, therefore, that there is a breakdown of the reserve proteins with the formation of the simpler soluble forms of nitrogen.

The decrease in the amount of non-protein nitrogen during the growing period, both in wood and in bark, is doubtless due to the rapid elaboration of complex nitrogen compounds by the developing tissues. From a supply of the simple nitrogenous compounds, proteins are synthesized in the leaves and appear as insoluble products. The total water soluble materials also show a decrease during this period.

The sudden increase in the amount of non-protein nitrogen in November immediately following leaf fall, may be related to the so-called autumnal migration of nitrogen from the falling leaves. This point is elaborated further in the discussion of the data on the nitrogen content of leaves.

It will be observed from Figs. 4B and 15B that not only in amount but as percentage of the total nitrogen, the total non-protein nitrogen is always greater in arable samples than in those from the grass area.

Total Non-Protein Nitrogen Fractions.

Ammonia (Figs. 5A, 5B, 16A, 16B). From the nature of the desiccation method adopted in the preservation of the sample—ammonia, present as such in the tissues, if any, is necessarily lost during drying. Ammonia, as determined, simply means ammonia present in the form of ammonium salts in the protein free extract.

Thomas (42) found that the ammonia fraction was usually very small except at one stage. Mulay (24) did not determine this fraction separately but included it with the acid amide nitrogen.

A study of Figs. 5A and 16A seem to show that this fraction is present in appreciable amounts only for a short period of the cycle. For the rest of the year, the amount of the fraction is quite low both in wood and in bark.

In wood, the ammonia content is high in the young shoots in June and then decreases steadily as growth progresses and, by leaf fall, has already reached a level when it is present in very small amounts. The curves for grass and arable samples are practically identical except in July. Expressed as percentages of the total non-protein nitrogen (Fig. 5B) the ammonia content is slightly higher in the case of grass samples, though the nature of the cycles is not essentially different.

In bark (Fig. 16A), the amounts of ammonia show more significant changes. The content reaches its peak in the case of arable samples in May, corresponding to the high amount of the total non-protein nitrogen at the time. The ammonia content in bark from the grass plot shows a rise in May and is at its maximum only in the new shoots. In both cases, there is a steady decrease with the progress of growth and a low level is reached in October. The content in the bark from the arable plot is distinctly higher than that in bark from the grass plot during the growing season.

Acid Amide (Figs. 6A, 6B, 17A, 17B). Wood—(Figs. 6A and 6B). The amount of acid amide present in wood is always higher in arable samples than in those from the grass plot—thus resembling the total non-protein nitrogen. Expressed as percentage of non-protein nitrogen, the fraction still shows higher values for arable samples. The figures for both arable and grass samples are similar. From a maximum at the commencement of shoot growth, the amount of acid amide decreases during the growing period and reaches a somewhat steady low level.

Bark—The amount of acid amide present in the bark is generally less than that present in the wood. A study of Fig. 17A brings out clearly the difference between the bark samples from arable and grass plots concerning this fraction. The curve for bark from the grass plot resembles closely the curve for ammonia. In the case of bark from the arable plot, however, the amount of acid amide, instead of decreasing steadily remains at a fairly high level throughout the growing period and registers a very rapid decrease at leaf fall. Fig. 17B shows that, at the time when acid amide nitrogen is at its highest as a percentage of non-protein nitrogen during the growing period in arable samples, the fraction has reached its minimum percentage for this particular period in bark from the grass plot.

Humin (Figs. 7A, 7B, 18A, 18B). The nature of substances determined under this fraction is not very clear. It is assumed that the black insoluble material to which the term "humin" is applied is formed during the acid hydrolysis as a complex reaction between mono and di-amino acids and carbohydrates. Gortner (9) and his associates have studied the formation of humin in detail.

The nature of the curves for humin content (Figs. 7 and 18A) appear, as a rule, to be similar to those for the total water soluble material. The formation of humin depends, according to the above view, on the presence of amino acids and carbohydrates. The latter constituents usually form a major part of the water soluble material and thus the parallelism found between the amounts of humin and total soluble matter is in agreement with the ideas on the origin of the humin fraction.

In bark, however, there is an increase in the amount of humin in May in place of a minimum as is obtained with water soluble material. There is also a corresponding increase in the amount of basic nitrogen at this period. These facts indicate the presence in fairly large amounts of mono and di-amino acids which are shown to be necessary for the synthesis of asparagine.

The amount of the fraction is always higher in the arable samples than in those from the grassed trees but the proportions in non-protein N are fairly similar (Figs. 7B, 18B).

Basic (Figs. 8A, 8B, 19A, 19B). Basic nitrogen as determined by the precipitation with phosphotungstic acid consists mainly of di-amino acids together with other nitrogen compounds of basic nature. Lysine, arginine and histidine are the three basic amino acids likely to be of importance in this fraction.

The basic nitrogen in wood (Fig. 8A) does not show any well defined changes other than a peak value in June, being otherwise low for most parts of the year.

The bark (Fig. 19A) undergoes marked seasonal changes in the amount of these basic nitrogen compounds. There is a rise, as explained previously in discussing the humin faction, in May, preceding new growth. A rapid decrease takes place and the minimum is reached by the end of the active shoot growth period. Subsequently a rise occurs through the full growth period and, after reaching a peak value at the end of November at defoliation, the amount slowly decreases.

Basic nitrogen compounds are very important in plant physiology and are structural units of nucleic acids. During the period of active shoot growth they may, therefore, be utilized for the synthesis of nucleo-proteids which constitute most of the nuclear contents.

The actual amounts of the fraction present are generally higher in the arable samples, but as a percentage of non-protein N the values in the bark are usually greater in the grass samples (Fig. 19B).

Imide (Figs. 9A, 9B, 20A, 20B). Imide nitrogen usually represents the nitrogen of heterocyclic compounds like proline, oxyproline and compounds with indol linkages. The figures for imide nitrogen in this investigation also include, as explained under chemical methods, the small quantities of mono-amino acid nitrogen determined. The fraction of non-protein nitrogen termed "Rest Nitrogen" by Thomas (42) or "Other Nitrogen" of Chibnall (3) included the imide fraction. Both Chibnall and Thomas considered that these nitrogen compounds were in some way connected with protein degradation.

Both in wood and in bark, the imide nitrogen forms a large part of the total non-protein nitrogen. In wood it ranges from 20 to 55 per cent. and in bark from 24 to 40 per cent. of the non-protein nitrogen during the seasonal cycle.

In wood (Figs. 9A and 9B), the imide nitrogen with one exception is much higher in arable samples than in those from grass. The curves for arable and grass are similar to those for total water soluble material and similar to each other in form. Expressed as a percentage of the total non-protein nitrogen, the curves are again usually similar in character and run quite close to each other. They show a minimum proportion in June and then a steady rise until the period of dormancy.

In bark (Fig. 20A), there is a steady decrease to November, an increase during that month and then the values for the fraction are steady. In the bark (Fig. 20B), the constituent, when expressed as a percentage of the total non-protein nitrogen, shows a decrease up to June, then a rise until the beginning of September followed by a decrease, and again a rise until the period of dormancy. The percentage of the imide nitrogen in non-protein N is, as a rule, higher in bark from the grass plot than in that from the arable area.

Rest (Figs. 10A, 10B, 21A, 21B). The values obtained for rest nitrogen in the wood (Fig. 10A) are somewhat erratic though the curves for the fraction for arable and grass samples are similar in form. The quantity is usually higher on the arable plot.

The amount of the constituent in the bark (Fig. 21) from the arable plot generally shows higher values than in the grass plot, but the cycles again appear similar in form. The value for the May grass plot sample appears abnormal.

Nitrate (Figs. 11A, 11B, 22A, 22B). Measurable quantities of nitrate nitrogen could not be detected up to June,* except in wood in May, and when the constituent was found, it was present in small quantities. No significance can be attached to the values at present, and they are presented without further comment pending further critical experimental work.

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* Since Dr. Karmarkar's departure qualitative tests for nitrate have been made on these samples and positive results obtained in all cases. (T.W.)

DATA FOR LEAVES.

The data for leaves refer to the time from which the first fully grown leaves were present to practically complete defoliation. On inspection of the data presented in Tables I and II it will be seen that the leaves from arable and grass plots do not differ significantly from each other at any time as regards water content or proportion of dry leaves to total dry shoots, including leaves. The water content ranges approximately from 64 to 60 per cent. over the season, being highest in the beginning and during defoliation in November.* The proportion of dry leaves to dry shoots prior to the defoliation period varies approximately from 75 to 45 per cent., decreasing steadily as growth proceeds. From these data it will again be evident that the main course of seasonal growth was similar on the two plots.

Total Nitrogen (Fig. 23).

The quantity of total nitrogen, expressed on a basis of dry matter, is highest at the beginning of the season, shows a sharp fall during rapid extension growth of the shoots to the end of July and then a gradual rise until the end of September, from which time a rapid and continuous fall occurs until defoliation in November.

The last stage of the curve is of special interest. It represents the so-called "Ruckwanderung" or autumnal migration of nitrogen from the leaves to the branches, which phenomenon has been a point of discussion by many workers. Combes (7, 8) has reported an extensive study of this subject in connection with young oak and beech trees. According to him, the migration of nitrogenous substances occurs as soon as the leaves turn yellow, at first slowly and then rapidly. The results of Thomas (42) indicated that the movement of nitrogen from the leaves was not initiated suddenly at the outset of yellowing but started much earlier. Lincoln (17) also observed the loss of nitrogen from pear leaves in association with natural defoliation.

The present investigation confirms the existence of the migration phenomenon, apparently commencing during October, possibly prior to the appearance of tinting, and progressing steadily and rapidly.

The picture of the migration is made still more clear when the curves for non-protein N in leaves and bark are examined. In the former case there is a sharp rise in the arable sample during October and in the grass sample a more steady and gradual rise during September and October, the final samples being taken in October; in the latter in both cases the fraction rises sharply during the period of defoliation in November.

---

* The leaves in the November sample were mainly younger leaves from the tip portions of the shoots.

Total Water Soluble Materials (Fig. 24).

The only feature concerning water soluble materials which requires particular mention is the steady fall in amounts in both cases throughout the period. The amount present in the grass sample is always higher than that from the arable plot.

Protein (Figs. 25A, 25B).

The curves for protein are essentially similar to those for total nitrogen, the amounts being higher in the samples from the arable trees. The percentage of the constituent in the total nitrogen only varies within very narrow limits, between 90 per cent. and 93 per cent. approximately.

Total Non-Protein (Figs. 26A, 26B).

The curves for the two plots vary somewhat over the season, but both show the interesting point of a rise during the tinting period. The constituent, as a percentage of total N, remains fairly constant over the season in both cases, varying between 6·5 per cent. and 10 per cent. approximately, and being very similar in the two cases. The relationship between the two plots differs in the leaves from those in the wood and bark, where the fraction in total amount and as a percentage of total N is distinctly higher in the arable plot. The difference in the two cases may be due to the rapid movement of soluble N products from the leaves throughout the season.

Total Non-Protein Nitrogen Fractions.

Ammonia (Figs. 27A, 27B). Both in actual amount and as a percentage of non-protein N, this fraction shows a more or less steady rise with the advance in the age of the leaves. The amounts present in the two cases are practically identical with the exception of the September values. The range of values as percentage protein N are similar to those in wood and bark.

Acid Amide (Figs. 28A, 28B). This fraction is at a minimum during August and September when the leaves and shoots were fully grown, prior to the onset of autumnal effects. The curves are thus dissimilar in form from those for ammonia and it will also be noted that the fraction is present in less amounts than ammonia, which is different from the conditions existing in the wood and bark. The fraction forms a very small proportion of non-protein N at all times.

The curves for the two plots are very close, slightly higher values tending to occur in the arable trees.

Humin (Figs. 29A, 29B). Humin N shows decided peak values, both in amounts and percentages of non-protein N, in July and August, during which period the quantities present in the arable trees are distinctly higher than those under grass. The percentages in non-protein N are similar in the two cases over the season. The constituent forms a considerable proportion of the total non-protein—25 per cent. to 36 per cent.—being second in amount to imide.

Basic (Figs. 30A, 30B). On the grass plot the values show a continuous fall over the period but on the arable area the amounts are rather irregular. As in the bark, the proportion of the constituent in the non-protein N is higher on the grass plot.

Imide (Figs. 31A, 31B). This constituent forms the largest percentage of the total non-protein N, the values ranging from 30 per cent. to 50 per cent. of the latter. There is one value missing for the grass samples but it would appear that for both plots there is a general tendency for the constituent to increase during the season, a well marked rise occurring during October.

The relationships between the values on the two plots are not very clear, those for the arable trees beginning at a higher level than those from the grass and finishing lower.

Rest (Figs. 32A, 32B). On both plots this category of nitrogen is only present in appreciable amount in the young foliage in June.

Nitrate (Figs. 33A, 33B). Only three determinations are available for the grass plot, but it would appear from these, in conjunction with the curves for the arable trees, that nitrate, after commencing at a maximum, falls steadily throughout the season to negligible amounts in September. The amounts are always very small.

CONCLUSIONS.

The main conclusions which may be drawn from the data obtained in the investigations are as follows:

1. Commencing from the dormant period, the water content of the wood and bark of terminal shoots rises through the periods of bud swelling and blossoming to reach a maximum in the young shoots in June. It subsequently decreases through the growing season to the period of autumnal tinting in October, eventually remaining at a fairly steady level during the period of dormancy.

In the leaves, two maxima and one minimum occur for water content, the former in the young leaves and during defoliation, and the latter during the period between the cessation of shoot growth and the onset of autumnal tinting.

2. The proportion of dry wood in the bark plus wood portion of the terminal shoots varies from about 25 per cent. at the time of the first fully grown leaves in June to approximately 50 per cent. in the fully grown shoots.

The percentage of dry leaves in dry shoots—wood, bark and leaves—varies from approximately 74 per cent. in June, when the first fully grown leaves are present, to about 44 per cent. in October immediately prior to defoliation.

3. The two cultural treatments, grass + nitrate in spring and arable without nitrate, did not alter significantly the percentage of water in the wood, bark and leaves portions of the shoots, nor the ratios of dry wood, dry bark and dry leaves in the shoots.

4. Well defined cycles occurred for total soluble materials, total nitrogen, protein N, non-protein N and the various fractions of the last of these in wood, bark and leaves.

5. In wood, bark and leaves portions of the shoots, the corresponding values for total nitrogen and protein N are similar from the two plots, though the amounts are higher in the arable plot samples. In wood and bark the percentages of protein N in total N are always higher in the grass samples, but in leaves the corresponding percentage values are practically equal for the two series.

In the case of non-protein N, the amounts in wood and bark are always higher in arable samples and the percentages of this constituent in total N are also invariably higher in these samples. In the leaves, clear cut differences are not found and the proportions of non-protein N to total N on the two plots are similar.

The results indicate a higher nitrogen status in the arable trees than in those on the grassed area.

6. The spring application of nitrate to the trees under grass does not appear to affect the character of the nitrogen cycles in the trees.

7. The cycles of nitrogenous constituents appear to be simpler in the wood than in the bark. In the former there is usually a very prominent peak value in June in the young shoots, following a distinct though relatively small fall during the period of bud swelling and blossoming. The June peak is followed by minor fluctuations and tends to remain at a lower level from July to the latter stages of dormancy.

In the bark, prominent peaks are also shown in June by the various fractions but in some cases fairly prominent peak values are also associated with other periods: total N, protein N, humin N and imide N with dormancy; and non-protein N, basic N and rest N with the onset of the dormant period in November.

In the wood and bark the significance of the values for nitrate N are not clear and it has not been established that this fraction as determined is "true" nitrate.

In the leaves, the general character of the cycles differs from those in wood and bark, and in this case outstanding features are the downward trends of the curves for total N and protein N, with especially significant falls during the autumn when nitrogen was undoubtedly passed from the leaves into the bark. The fall in these constituents is associated with a rise in the non-protein N fraction in the leaves and concurrently with rises in total N, protein N and non-protein N in the bark.

8. Comparison of the amounts of total N in wood, bark and leaves shows these to be in the order wood < bark < leaves, whilst the percentages of protein N in the total N are in the same order, and conversely the percentages of non-protein N in the reverse direction.

9. The relative proportions of the various N fractions comprising the non-protein N are fairly similar for wood and bark but the parallelism with these in the leaves is not so close, the fractions ammonia, acid amide and rest N differing from the proportions found in the other two cases.

The composition of the non-protein fractions in the corresponding cases is similar in the grass and arable samples, thus showing that the composition of the non-protein N is not radically affected by the two levels of nitrogen nutrition obtaining under the two cultural systems.

10. The salient points requiring mention in connection with the various fractions of non-protein N are as follows:

Wood and Bark. In the wood the most prominent portions are imide, acid amide, humin and rest N, the fractions, ammonia, basic and nitrate N, especially the last one, only being present in small amounts. The proportions in the bark are fairly similar to those in the wood with the exception that basic N is of more importance.

In wood and bark, ammonia N and acid amide N form considerable percentages of non-protein N only during the active growing period of the shoots. Humin N is always present as a fairly large percentage of non-protein N and does not show marked periodic trends.

Basic N in non-protein N in the bark shows a marked minimum value from midsummer to September. The higher percentage values for the grass samples than for the arable are notable.

Imide N, as percentage of non-protein N, tends to show minimum values in both wood and bark at the period of most active growth in June.

The percentage values for rest N in non-protein N are somewhat irregular, especially for the grass samples, but they indicate a steady fall in the wood from the time of bud swelling right through the growing season into the dormant period, whilst in the bark a minimum value is suggested at the end of the active shoot elongation period in July or August.

Leaves. In the leaves imide N is again present in the greatest quantities but humin N is also very abundant. Basic N is the only other fraction present in fair quantity, all others being especially low in amount, rest N being notably so.

Ammonia N and acid amide N do not show similar cycles as in wood and bark, the former rising steadily with the age of the leaves whilst the latter shows highest values at the beginning and end of the foliation period and minimum values in August and September.

Acid amide in the leaves is lower than ammonia.

Humin N is at a maximum during the period of full growth in July and August, when acid amide is at a minimum.

Basic N shows a steady decrease in the grass samples but fluctuates somewhat in the arable ones. The amounts are usually higher in the grass than in the arable samples.

Imide N rises with the advance in the age of the leaves, maximum values occurring near the defoliation period.

Rest N is negligible in amount except in June.

Nitrate N is only present in appreciable amounts in June, falling steadily throughout the season to very low values.

SUMMARY.

1. An investigation is described in which the seasonal cycles of total nitrogen and of various fractions on non-protein nitrogen in the terminal shoots of apple trees, var. Newton Wonder, have been followed.

2. Two sets of trees, under different cultural conditions, were included in the investigations in order to determine the effects of the two systems on the nitrogen status in the two series. In one case the trees are growing under arable conditions without nitrogenous manuring and in the other a system of grass culture with annual dressings of nitrate in spring is followed.

3. Samples of terminal shoots, consisting of 50 to 60 shoots each, from the two plots were collected at monthly intervals from March 1932 to March 1933. They were always taken between 10.30 a.m. and 11.30 a.m. to avoid diurnal variations.

4. For the purposes of analysis the shoots were divided into the portions wood, bark and leaves.

5. The determinations made were: mean lengths of shoots; total dry matter of shoots and of wood, bark and leaves; total water soluble materials; total nitrogen, protein N and non-protein N; fractions of non-protein N,

including nitrate, ammonia, acid amide, humin, basic, imide (including mono-amino acid) and rest N.

6. The various constituents determined were found to exhibit definite seasonal cycles in all three portions of the shoots. The forms of the cycles of the respective constituents in wood and bark were often similar, but those in the leaves differed from these.

The results for leaves and bark during the period September to November demonstrated the phenomenon of autumnal migration.

7. The application of nitrate in March to the trees under grass culture did not affect the form of the nitrogen cycles in the trees, and the data for the two series indicate a higher nitrogen status within the trees on the arable plot than on the grassed area.

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APPENDIX.

CHEMICAL METHODS.

Total Nitrogen. For this purpose 0·3 to 0·4 gms. of the material is employed. The aliquot is placed in the Kjeldahl flask, 0·5 gm. of Devarda alloy and 100 c.c. of N/10 NaOH are added together with a little paraffin wax to reduce frothing. Nitrates present are reduced by distilling for one hour into standard sulphuric acid (N/50). Organic nitrogen is then liberated by digesting with 25 c.c. of sulphuric acid and 10 gms. of potassium sulphate. The distillation is completed into the same flask of standard acid. The distillate in this and other estimations is always boiled and cooled previous to titration to secure a sharp end point.

Extraction of the Non-Protein Nitrogen. From 30 to 40 gms. of the dry material is weighed out in cylindrical bottles of 500 c.c. capacity, 200 c.c. of ammonia-free distilled water added, the bottles well corked, well shaken for five minutes and then allowed to stand for half an hour with frequent shaking. The contents are then well shaken again and pressed through a filtering cloth. The pressed residue from the cloth is again transferred back to the bottles, 100 c.c. of water added, well shaken and allowed to stand fifteen minutes. The contents are filtered and pressed as before and the whole process repeated two more times. Finally the filtrates are bulked and filtered through paper pulp at the filter pump. The clear filtrate obtained is then brought to boiling point and acidified with a few drops of acetic acid. This is cooled and set aside for a few hours to allow any coagulated matter to settle out. The extract is then filtered through an ordinary filter paper and made up to 500 c.c. A few drops of toluene are added as a preservative.

Total Water Soluble Matter. 25 c.c. of the extract are evaporated on a water bath and then dried at 70° C. to constant weight.

Total Non-Protein Nitrogen. 25 c.c. of the extract are used for the determination of this fraction. The procedure described for the estimation of total nitrogen is followed.

Nitrate Nitrogen. 50 c.c. of the extract are treated with 5 gms. of reduced iron and 10 c.c. of 1:3 sulphuric acid. This reduction is carried out in conical flasks stoppered with gas locks. After the effervescence has ceased, the contents are washed out into a distilling flask, made alkaline with 5 gms. of MgO and ammonia determined as described below. Nitrate nitrogen is obtained by subtracting the amount of free ammonia from the amount of ammonia determined after reduction.

Ammonia. 50 c.c. of the extract are made alkaline with a paste of 2 gms. of MgO, some water added and then distilled under reduced pressure at about 40° C. into standard acid.

Acid Amide. 100 c.c. of the extract are hydrolysed under a reflux for two hours on a boiling water bath with 4 per cent. hydrochloric acid and the acid driven off by vacuum distillation. The residue is washed out, transferred to a distilling flask, made alkaline with MgO and the ammonia content determined. The amount of ammonia thus determined minus the amount of free ammonia gives the amount of acid amide nitrogen.

Humin Nitrogen. After the distillation, the contents of the distillation flask are completely washed out and filtered. The residue of MgO with the humin formed during hydrolysis is repeatedly washed with hot water. The residue is air dried, which prevents frothing, and then Kjeldahlized along with the filter paper. (In all cases a blank determination was made.)

Basic Nitrogen. The filtrate from the MgO residue is then concentrated in vacuo to 100 c.c. and cooled. 30 c.c. of 20 per cent. phosphotungstic acid solution (containing 5 per cent. H_2SO_4) are added after the addition of 5 gms. of sulphuric acid and the precipitate formed allowed to settle overnight. The precipitate is subsequently decanted, transferred quantitatively on to the filter paper and thoroughly washed with about 200 c.c. of a solution of 2·5 per cent. phosphotungstic acid containing 5 per cent. sulphuric acid. The precipitate along with the filter paper is then Kjeldahlized.

Imide Nitrogen. The filtrate from the above is neutralized with pure calcium carbonate and the precipitated calcium sulphate filtered off and washed. This preliminary removal of sulphuric acid was found somewhat convenient as it avoids the formation of a large amount of barium sulphate precipitate which is rather difficult to filter. The filtrate after the separation of $CaSO_4$ is warmed and barium hydroxide added for complete removal of phosphotungstic acid. The precipitate obtained is again filtered off, well washed, and the excess of barium hydroxide in the filtrate removed by treating it with CO_2 gas. The filtrate from barium carbonate precipitate is then evaporated in vacuo to about 80 c.c., 2 gms. of barium chloride added to remove any phosphoric acid and after filtration made up to 100 c.c. 25 c.c. of this solution are then Kjeldahlized. This amount also includes the mono-amino-nitrogen determined as given below.

Mono-amino Acid Nitrogen. 25 c.c. of the above solution are formol titrated according to Sorensen's method, N/50 barium hydroxide being used. At this stage the solution has only a pale yellow colour and therefore no difficulty is encountered in the titration.

Rest Nitrogen. The total non-protein nitrogen minus the sum total of ammonia, nitrate, acid amide, humin, basic and imide (including mono-amino acid) nitrogen fractions gives the amount of rest nitrogen.

Proteose Nitrogen. This was determined as an outside figure and has, therefore, no place in the systematic fractionation. 25 c.c. of the original extract were made slightly acid by means of a few drops of dilute sulphuric acid, saturated with zinc sulphate and then left to settle overnight. The coagulum obtained was filtered and washed two or three times with a saturated solution of zinc sulphate. It was then Kjeldahlized along with the filter paper.*

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* The results obtained for this fraction are not given in this paper since it is considered that further work is necessary to increase the accuracy of the determinations.

ROOT ROTS OF STRAWBERRY IN BRITAIN

THE "BLACK LESION" TYPE OF STRAWBERRY ROOT ROT

By G. H. BERKELEY,* M.A., Ph.D.,

Senior Pathologist-in-charge, Dominion Laboratory of Plant Pathology,
St. Catharines, Ontario, Canada, and

ISABEL LAUDER-THOMSON, B.Sc.,
East Malling Research Station, East Malling, Kent

INTRODUCTION.

In recent years considerable attention has been given to the question of the degeneration of the strawberry because of the numerous and increasing difficulties encountered by the growers of this crop. In the United States of America, Canada, Australia, Britain and more recently France and Holland, deterioration or degeneration of the strawberry has become increasingly apparent and important. This condition has become so widespread that The Imperial Bureau of Fruit Production, East Malling, has recently published a résumé (1) of the literature dealing with "degeneration" from the standpoints of (a) pomology, (b) virus, (c) root rot and (d) insects.

In many localities in the United States of America and Canada, and in the Clyde Valley, Scotland, "root rots" are a factor of great importance, and are considered by many to be one of the fundamental causes of plant failure in strawberry cultivation. In England the possibility of "root rots" being a contributory factor in the degeneration of the strawberry, has been largely overlooked. Recently, however, Berkeley (5, 6) working at East Malling demonstrated that "root rot" of the black lesion type is present in England.

Strawberry diseases in Britain have received considerable attention during the last ten or twelve years. Although much information has been obtained and many diseases have been recognized as a result of these studies, it must be admitted that the problem is still far from solved, only partial remedies for particular aspects having been evolved. In 1927 Ball, Mann and Staniland (4) classified the various forms of strawberry degeneration into seven groups, with provisional causes assigned. They did not however include "virus" or "root rot" as possible factors. Recently Harris (11) has shown conclusively that virus plays a part in this degeneration, and Berkeley (5, 6) has brought forth evidence suggesting that root rots also are of importance in this connection. The disease described by Mann, Ball and Staniland as "Aphis plant", the symptoms of which they state are similar to Xanthosis as described by Plakidas (14) is most likely the yellow-edge disease recently reported by Harris, while the diseases described by them as "patch", "sudden wilt" and "little leaf", may well be the "root rot" disease complex described in this paper. Root rot occurs in a patchy manner throughout a plantation (Fig. 1); badly affected plants wilt (particularly at fruiting time) and are dwarfed, with small leaves and petioles, suggesting the names "patch", "sudden wilt" and "little leaf" (Fig. 2) as used by Mann, Ball and Staniland. Whether or not any of the symptom-diseases already recorded in the literature for England are to be considered as due to root rot, may be problematical, but the fact remains that root rot diseases have been in England for some years though their true nature has not been understood until recently. It is true that the importance of these root rot diseases under English conditions has yet to be ascertained, but there is very good reason to believe that they play a major rôle in the general deterioration of the strawberry here in England, as they have already been demonstrated to do in the United States of America and Canada.

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* Worked at East Malling from May 26th, 1933, till June 1st, 1934.

Strong and Strong (15), reporting investigations on black root (root rot) of strawberries in Michigan, say "there is a situation in strawberry culture which is a subject of concern to the growers. This consists in the failure of plants newly set out to become established, and, often during the picking season, the dying of plants already established. In severe cases this latter condition may be so extensive as to render the plantation unprofitable. Losses in stand of plants in Michigan plantations have been observed to range from 5 per cent. to as high as 60 per cent. in some cases with subsequent reduction in crop yields."

In 1924 Berkeley (7) reported that "many plantations in Ontario, Canada, were severely attacked with root rot. . . . It was again observed that new settings suffered severely". Again in 1927 (8) he reports that "from the third week of June strawberry patches seemed to suffer most, even young patches suffered a heavy toll". This disease is considered to be most important under Ontario conditions since heavy losses have been encountered in some seasons. Individual plantations have been so attacked that what appeared to be a promising plantation in May or June was, later in the season, ploughed under as being unprofitable.

In 1932 Zeller (18) described a Rhizoctonia "root rot" and reported that "in some plantings, few plants with definite symptoms may be found, while in others high percentages of plants in extreme conditions of wilt may result. In one extreme case in a six acre planting of two-year-old Marshalls, 97 per cent. of the plants were wilted in June, but the remaining 3 per cent. showed severe root infection. . . . In general, Rhizoctonia root rot may be said to be one of the chief factors causing variability and unevenness in Marshall strawberry plantings in Pacific Northwest."

The above quotations clearly point out that root rots are considered by these authors to be a factor of considerable importance. In fact, wherever "root rots" of the strawberry have been studied the conclusion has been reached that they are a fundamental factor in connection with the deterioration of strawberry plantations. As a result of observations and study during one season at East Malling, we are of the opinion that "root rots" are fairly widespread in England, and that they are responsible for many patchy, uneven and unthrifty plantations. In this connection the following quotations from letters received from growers are of interest. A grower from Essex who sent plants in for diagnosis wrote, "My plants seem to do well the first year, but go off in the second and third years." A grower from Sussex wrote, "Paxtons do all right during the first and second years, but go off in their third. The young plants show signs of a bad fungus attack, but are sending out vigorous young roots above those dead; this appears to happen for two years, but not the third." A grower in Kent wrote, "The enclosed Oberschlesien plants were planted a year ago and they have practically died this winter. I see that the roots are in a bad condition. I wonder if they are suffering from foot rot." The plants sent in by the above growers showed in every case a severe root rot attack. Since no other disease was present it is concluded that root rot was the main factor in the above plantations.

A preliminary survey made by the senior author demonstrated that root rot was present in most plantations visited. In Hampshire several plantations were visited in which a large percentage of the plants were stunted with the dwarfed leaves and petioles associated with root rot diseases. In two such plantations root rot was undoubtedly the main, if not the sole cause of degeneration. In a few plantations no indications of root rot could be found, indicating that though the disease may be fairly widespread it is by no means present in all plantations.

In addition to Hampshire, root rots were readily found in Somerset, Sussex, Wiltshire, Dorset, Kent, Gloucester and Surrey. In Sussex and Kent, where the greatest number of plantations were visited, root rots were quite commonly found, and in numerous plantations they appeared to be the main disease.

This survey also pointed out that the Paxton variety was perhaps the most susceptible variety, with Royal Sovereign as a close second. Other varieties found attacked were Madam Kooi, Oberschlesien, Brenda Gautrey, Madam Lefebvre, Ruskin and Huxley's Unknown.

Through the kindness of Mrs. N. L. Alcock and Mr. D. V. Howells, the senior author was enabled to see the red-core type of root rot as found in the Lanarkshire district in Scotland. The symptoms of this red-core root rot are sufficiently different from those of the black lesion type to warrant considering these two types of rot, at this time, as distinct diseases. Acording to Mrs. Alcock

(2, 3) the red-core disease is caused by a species of *Phytophthora* which attacks the root system first by way of the root tips, and then the stele, whence it extends to the cortex. In this case the parasitic attack is from the centre of the root outwards, while with the black lesion type of root rot the attack is from the outside, that is, from the cortex inwards to the stele. The red-core symptom is not found in the black lesion type of root rot, while the black lesion is not a symptom of the *Phytophthora* root rot.

DISTRIBUTION.

According to the literature, strawberry root rots would appear to have a very widespread distribution. To date definite reports of diseases of this type have been made in (1) several states of the United States of America, (2) Canada, (3) Scotland, (4) England, (5) Australia and (6) France. Also from correspondence or information obtained first-hand, we are able to report root rots of strawberry as being present in Germany, Holland, New Zealand and South Africa. It would appear, therefore, that "root rot" diseases are present wherever strawberries are grown.

LITERATURE.

The literature dealing with root rots of strawberry has recently been summarized by the senior author (1) who gives thirty-one citations. There is, therefore, no necessity to present the literature in any detail in the present paper. It might be mentioned, however, that this literature is by no means extensive, since only in a comparatively few cases have fungi been proved to be a cause of "root rot". It does, however, conclusively demonstrate that parasitic diseases of the root rot type occur in widely separated countries, and that these diseases are considered as an important factor in the general present day "degeneration" of the strawberry. This literature also suggests that though there may be more than one type of root rot, for example, the "red-core" and "black lesion" types, at least one of these (the "black lesion" type) may also be caused by more than one fungus. In this connection *Coniothyrium Fuckelii, Hainesia Lythri, Ramularia* sp. (=*Cylindrocarpon radicicola* Wr.), and *Rhizoctonia Solani* have been reported as causal organisms of diseases of the "black lesion" type.

PRELIMINARY TESTS AS TO ETIOLOGY.

As a result of the survey there seemed to be no doubt that strawberry root rot was present in England. The next step, therefore, was to ascertain if our diagnosis of symptoms as seen in the field was correct. If such plants were affected with root rot then it should be possible to isolate from the diseased root system the fungus or fungi responsible, and to prove their pathogenicity by direct inoculation tests. Accordingly isolation and inoculation studies were started which have ultimately proved that root rots of the "black root" or "black lesion" type identical with those reported in America and elsewhere are present in many counties of England, and are undoubtedly important in connection with the general deterioration of strawberries in England.

When plants suspected to be affected with root rot were first observed and before any fungi had been isolated from diseased roots, the possible nature of the trouble on such plants was ascertained by inoculations made in sterilized soil with a root maceration of diseased roots, and by tests with sterilized and with unsterilized contaminated soil.

(a) Root Macerations.

In several preliminary tests, unrooted strawberry runners were struck in sterilized soil to which an infusion of macerated "lesioned" roots from naturally infected plants had been added as inoculum. Within a month definite "rotted" and "lesioned" roots were conspicuous on the inoculated plants while the root systems of the uninoculated control plants were white and healthy (Fig. 3). These tests strongly suggested a parasitic attack on the root system.

(b) Effect of Soil Sterilization.

In view of the above results with "root maceration" it seemed advisable to find out whether or not the organism or organisms responsible were also present in the soil itself. To this end unrooted runner plants were struck in sterilized and unsterilized naturally infected soil. In this case also after a month the root system that had formed in the unsterilized contaminated soil was considerably diseased with lesions and dead roots, whereas the root system that had formed in the sterilized soil was perfectly healthy.

These two tests confirmed our suspicions in that they definitely pointed to the cause of the trouble as being in the soil, and suggested it to be of a parasitic nature. The next step, therefore, was to isolate the fungi in pure culture and prove their pathogenicity by direct inoculation tests.

ISOLATIONS.

Perhaps the most difficult phase in the study of "root rots" is that concerned with securing isolations from the delicate root tissues. The roots are so small and tender that sterilization with chemicals such as mercuric chloride to be effective in killing the surface bacteria, often penetrates the entire root and kills everything. On the other hand, if no sterilization is effected and the roots are insufficiently washed, far too much bacterial growth will result. Therefore this phase of the problem received special study and the following methods were used, with methods (A) and (C) giving the best results. It was found that a prime essential was a thorough washing of the root tissues in a strong stream of running water followed by a rinsing in sterile distilled water.

METHOD A. FLASK METHOD.

Pieces of lesioned root were thoroughly washed in a stream of running water under a tap, and then suspended in a 250 c.c. flask of sterile distilled water to which two drops of lactic acid were added to restrain bacterial growth. In a few days such roots showed a profusion of fungal growth from which transfers could be readily made to culture tubes. Such transfers were by no means pure—in fact seldom so—but by the usual purification methods pure cultures of various fungi were obtained.

METHOD B. DIRECT STERILIZATION METHOD.

This consisted in sterilizing lesioned roots in 0·1 per cent. mercuric chloride for half a minute, cutting away the lesion with a portion of healthy root attached, and directly transferring to culture tubes. This method was satisfactory with the larger roots, but not for the smaller lateral roots, since sterilization of such roots by this method often resulted in no growth of any kind.

METHOD C. THE "IMMERSION IN AGAR" METHOD.

This method consisted in placing thoroughly washed and dried pieces of diseased roots in nutrient agar in a Petri dish, in such a way that the root-pieces were *completely covered* with the agar when it solidified. Success with this method depended upon (1) thorough washing of the root-pieces and (2) placing the root-pieces in the agar before it solidified.

Sometimes when the roots were insufficiently washed, bacteria appeared on the surface of the agar just above the pieces of root. When this happened it was found that the bacteria could be destroyed by washing the surface with a weak solution of mercuric chloride, without killing any fungi that might be present in the root tissue.

This method gave the quickest and, on the whole, the best results in that pure cultures were commonly obtained with it. For instance, in twenty-five to thirty minutes, ten Petri dishes may be set up with four pieces of root in each, making forty isolations in all. In one such case, each of the forty pieces produced a growth of *Cylindrocarpon radicicola* showing the degree of efficiency that was sometimes obtained. It is not to be inferred, however, that results were always of this order, because more often they were not; nevertheless, consistently good results were obtained with this method.

Material for Isolations.

Isolations were made direct from diseased root tissue as brought in from the field, or preferably by an indirect method using inoculated material as follows. Naturally infected and lesioned roots were cut up into fine pieces, and mixed with sterilized soil in a pot. Then unrooted runner plants were struck in this soil, so that the entire root system would form in the root-inoculated sterilized soil. Isolations were then made from the inoculated lesions which formed on the new root system. This method gave fewer contaminating fungi and was the method used in all the early isolations.

Therefore by means of this material and one of the above methods, isolations were made from diseased strawberry roots throughout the growing season of 1933 and during the winter months that followed.

Species of the following genera were isolated: *Coniothyrium, Hainesia, Cylindrocarpon* (= *Ramularia* previously reported), *Fusarium, Pachybasium, Phoma, Botrytis, Chalaropsis, Penicillium, Rhizopus, Verticillium, Sphaeropsis, Cephalothecium, Rhizoctonia* (the orchid type), *Pythium* and several other Phycomycetes, and an unidentified sterile fungus. Nematodes also were sometimes obtained. It is, of course, obvious that many, if not most, of these fungi are saprophytic in the dead cortex infected by the root rot organism. This is borne out by the fact that most of these fungi gave negative results in inoculation tests, and also that microscopic examination of free hand sections of lesioned roots showed the presence of several types of mycelium indicating the presence of several fungi. In many roots a mycorrhizal fungus similar to that described by O'Brien and McNaughton (13) was observed as well as mycelium which varied from fine to coarse, septate to nonsepate, and colourless to dark brown. Also chlamydospores and pseudo-sclerotial groups were often present. This great variety of fungous flora in the cortex of lesioned roots suggests that much of it is secondary in nature.

An interesting observation in connection with the series of isolations was that different fungi were isolated at different times of the year, and that the fungous flora from one district was not necessarily the same as that from another. For instance, the early isolations in 1933 gave rise to *Coniothyrium, Fusaria, Rhizopus* and an occasional *Cylindrocarpon,* whereas later in the season *Cylindrocarpon* was isolated much more frequently, and *Coniothyrium* less frequently. Cultures of *Hainesia* were not isolated in the early part of the season and the first isolation was not obtained until late September. It is also interesting to note that *Coniothyrium, Cylindrocarpon* and certain *Fusaria* were isolated throughout the season.

Pachybasium was isolated from a single locality in Surrey. *Coniothyrium* was isolated from Hampshire, Sussex and Kent, but not from Wiltshire or Scotland. *Hainesia* was isolated from the Clyde Valley, Scotland, and Kent, England. *Cylindrocarpon* and *Fusarium* sp. were isolated from Kent, Hampshire, Somerset, Wiltshire, Sussex and Clyde Valley, Scotland.

On two or three occasions, especially during the early spring, numerous nematodes were found in the fine roots of a few plants. Also on a few occasions isolations from pieces of "lesioned-roots" in a Petri dish gave rise to numerous nematodes. The possibility of nematodes being a factor in the root rot complex should not be overlooked.

EXPERIMENTAL STUDIES WITH PURE CULTURES.

Inoculation Tests.

It was soon realized that a quick preliminary test for pathogenicity would be of great advantage in view of the large number of apparently different fungi that had been obtained. Several methods were tried, and the following one was chosen as it gave consistently good results. First, unrooted runner plants from a healthy Royal Sovereign clone were struck in 3 in. pots. These plants were prepared considerably ahead of time, and when the root systems had become so extensive that upon carefully knocking the plants out of the pots, they came out as a solid compact ball of roots and soil, they were ready for inoculation. Inoculations were effected by placing a small piece of the fungous culture (mycelium + agar) on an exposed bit of root, covering it with a very thin layer of moist absorbent cotton wool, tying the cotton wool in place with thread, and then replacing the compact root system in the pot. In this way several inoculations could be made on a single plant, and a large number of isolants could be tested in a short time, because it was found that the parasitic organisms produced complete collapse of the inoculated roots in from three to ten days. The essential point with this technique is to keep the cotton wool pad moist. To do this the soil must be kept slightly damp and not be allowed to dry out. This method was therefore used with all isolants in an endeavour to ascertain in a short time their parasitic tendencies. When a fungus produced rotting, or even pronounced discoloration of the inoculated root (sterile agar as a check produced no discoloration whatever) it was then tested on a much more comprehensive scale as follows.

The fungus was obtained in pure culture and then grown in flasks on sterile oat hulls.* These oat hull cultures were then mixed with sterilized soil in pots, and new unrooted runner plants were allowed to strike in this inoculated soil. Oat hulls are ideal for this purpose as they readily mix with the soil to give an even distribution of inoculum. Plants inoculated in this way were allowed to grow for some two to three months or until the above ground symptoms of dwarfed and stunted foliage became apparent. Under greenhouse conditions, where many of these inoculations were carried out, the first indication often was a yellowing of the lower leaves followed by dwarfing of the foliage generally.

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* Oat hulls also known as oat cavings or chaff.

In this way *Coniothyrium* sp., *Hainesia* sp., *Cylindrocarpon* sp., *Fusarium* sp., *Pachybasium* sp., all proved to be capable of producing root rot of strawberry.

Before a fungus was considered to be parasitic its pathogenicity was demonstrated by the above method in at least two different experiments and the results of inoculation had to be clear cut, that is, the inoculated plants had to show many dead and lesioned roots, while the root system of the check plants had to be perfect before they were accepted as proof of pathogenicity. As each of the above five fungi has been demonstrated under experimental conditions to be capable of causing "root rot" on strawberry, and as these fungi were isolated from different districts, at different seasons of the year, singly and in various associations, it has been considered preferable to treat each as the cause of a distinct root rot disease, even though the symptoms of all five root rots may be the same. It should be specially pointed out here that in so far as general symptoms are concerned, these diseases cannot be separated in the field since the same set of symptoms—dwarfed, stunted, plants, small leaves and petioles, with " black lesions " on roots, rotted roots and absence of fibrous roots—are common to all of them.

Though at the present time we are inclined to the view that all the root rot diseases herein discussed have common symptoms, there is some circumstantial evidence which suggests that a superficial cortical rotting may be associated with a specific fungus or fungi. This possibility needs to be investigated further.

The five diseases will now be discussed individually.

1. CONIOTHYRIUM ROOT ROT (*Coniothyrium Fuckelii* Sacc.).

This root rot of strawberry was first described in Michigan, U.S.A., by Strong and Strong (15) in conjunction with *Hainesia Lythri*, as the cause of a "black root" condition of strawberry roots. They considered the fungus responsible for this root rot to be the raspberry cane blight organism, *Coniothyrium Fuckelii*, as the spore size and general pycnidial characters were in close agreement with those of this species. They did not carry out cross inoculations studies however.

Distribution. In Michigan, as already pointed out, *Coniothyrium* root rot is widespread and considered to be of considerable economic importance. From unpublished work the senior author is able to say that *Coniothyrium* root rot of strawberry is also present in Ontario, Canada. During this past season this same root rot has been found in England in Kent, Sussex, Hampshire and Somerset. In Kent it seems to be fairly widespread, is often associated with other root rot organisms and is undoubtedly important in the deterioration of strawberry plantations.

Symptoms. The symptoms of this root rot have already been given by Strong and Strong for Michigan conditions, and so far as we have been able to ascertain, the symptoms are similar for England.

Plants inoculated with *Coniothyrium Fuckelii* began to show a stunted type of growth in about six to eight weeks. The leaves were much smaller than those of the healthy check plants and the petioles were much shorter. The lower leaves wilted and died. Sometimes the first indication of root rot under greenhouse conditions was a yellowing of the lower leaves. Under outdoor conditions this yellowing is generally absent unless the plants are growing in wet soil, when it is sometimes present. The roots of plants affected with *Coniothyrium* root rot generally showed distinct lesions on the larger roots and a large percentage of the smaller roots and fine laterals were rotted away. In comparison the roots of a healthy plant were white and free from all lesions and rot.

Etiology. Isolations from material from Hampshire and Kent first gave a fair percentage of *Coniothyrium* cultures, 50 per cent. being obtained in some series of isolations. Although *Coniothyrium* cultures were obtained throughout the year from diseased roots from Kent yet a greater number were isolated during early summer, suggesting a greater activity on the part of this fungus at this time of the year. This is also borne out by the fact that inoculation tests carried out in the autumn were much slower in giving results, and, in fact, often no clear cut rotting of roots was obtained at this time. In the spring of the year, however, we have had no difficulty whatever in repeatedly obtaining definite cases of root rot with the *Coniothyrium* isolants.

That *Coniothyrium* is capable of producing root rot of strawberries in England has been conclusively proved by repeated inoculation tests. Some fifty plants have been inoculated at different times with this fungus with clear cut results. The inoculated plants showed definite root rot while the check plants remained healthy (Fig. 5D).

It should be pointed out here that virus-free, insect-free plants were used for these tests and that most of the inoculations were made by striking unrooted runner plants into oat hull inoculated soil. One series was effected with transplanted plants, the long roots of which were pruned before planting. In this case also the transplanted plants had been first struck in sterilized soil in pots as unrooted runner plants, and were later transferred into the oat hull inoculated soil.

In one case where transplanted plants, as above, were inoculated on October 19th, 1933, the roots had been actually rotted away by April 1934 except for a few roots that had grown along the side of the pot (Fig. 6).

In another experiment, *Coniothyrium* cultures from Michigan, U.S.A., Ontario, Canada, and England were compared. We are grateful to the Laboratory of Plant Pathology, Ontario, Canada, for supplying us with the Michigan and Ontario *Coniothyrium* cultures. A *Coniothyrium* culture from raspberry canes was also tested on strawberry roots.

TABLE I.

Comparison of Coniothyrium *cultures.*

Coniothyrium.	No. of plants inoculated.	No. definite root rot.	No. healthy.
Michigan	5	5	0
Ontario	5	5	0
England	5	5	0
Raspberry	5	5	0
Check (five plants) .. No inoculum	–	0	5

This test demonstrates that the culture from raspberry is capable of attacking strawberry roots. Therefore as the spore size and general characteristics of the strawberry isolant are within the range for *Coniothyrium Fuckelii* we follow the lead of Strong and Strong and consider this strawberry root rot fungus to be *Coniothyrium Fuckelii* Sacc.

TABLE II.

Inoculation Tests with Coniothyrium Fuckelii *from English strawberries.*

Coniothyrium Fuckelii.	No. of plants inoculated.	No. showing root rot in two months.	No. healthy.
Kent	20	20	0
Hampshire	5	4	1
Check (five plants) ..	–	–	5

It might be well at this point to reiterate that positive results from inoculations were based on the presence of definitely rotted roots, or roots with distinct black lesions, in comparison with perfectly normal and healthy roots of control plants.

The evidence given here definitely demonstrates the presence of *Coniothyrium* root rot in England.

2. HAINESIA ROOT ROT (*Hainesia Lythri* (Desm.) v. Höhn.).

As already indicated this root rot was also first reported by Strong and Strong (15) for Michigan, as a partial cause of the "black rot" disease of strawberries.

Distribution. In addition to Michigan we can now add Lanarkshire, Scotland, and Kent, England, since we have isolated this fungus from diseased roots from both counties. It is interesting to note, however, that this fungus was not isolated from any material until late in the season, much later than the first *Coniothyrium* cultures. This in itself suggests the possibility of a sequence in root rot attack ; that under natural conditions *Coniothyrium* may be more active in the spring, and *Hainesia* later in the season. It would be interesting to know, if such a sequence does exists and, if so, whether it is related to temperature, moisture, or some other factor. *Hainesia Lythri* was not isolated from strawberry roots obtained from Hampshire, Wiltshire or Somerset.

Symptoms. Similar in all respects to the *Coniothyrium* root rot.

Etiology. This fungus was isolated only on three or four different occasions, so we know very little about its general prevalence. It was first isolated from strawberries sent in from Scotland in September 1933. Later it was isolated from different diseased root samples obtained in Kent. In culture our fungus is similar to the culture received from Michigan, and as spore size and shape are also in agreement, we have named the British Hainesia also *Hainesia Lythri*.

Proof of pathogenicity has been obtained repeatedly by the preliminary method and also by oat hull inoculations. In this connection it is particularly interesting to note that the *Hainesia Lythri* isolated from Kent has so far given us negative results with the preliminary method, though with the oat hull method diseased roots have been produced. On the other hand, the Scottish *Hainesia Lythri* has never failed to produce root rot with either method.

TABLE III.

Comparison of Michigan and British Hainesia Lythri.

Inoculum.	No. of plants inoculated.	No. of plants with root rot.	No. healthy.
Hainesia Scot. ..	5	5	0
Hainesia Mch. ..	5	5	0
Check plants (no inoculum, five) ..	–	0	5

There appears, therefore, to be no doubt that *Hainesia Lythri* is the cause of a root rot in Britain as well as in America. We have isolated it from different samples sent to us from Lanarkshire, Scotland, and from diseased roots at East Malling.

3. CYLINDROCARPON ROOT ROT (*Cylindrocarpon radicicola* Wr.).

This is most likely the root rot reported from Ontario, Canada, by Walker (17) as being caused by a *Ramularia* sp. A comparison of the Canadian and

English cultures has definitely shown that the organism in both cases is similar and that it should be called *Cylindrocarpon radicicola* Wr. We are greatly indebted to Dr. W. Wollenweber for identifying this fungus.

Distribution. The *Cylindrocarpon* root rot was first reported from Ontario, Canada. We have to report it for Kent, Sussex, Hampshire, Somerset and Wiltshire in England and Clyde Valley, Scotland. In this connection it is interesting to note that recently this same fungus has been reported as the cause of a root rot of narcissus and lily in Holland (10, 16).

Our isolations suggest that the fungus has a much wider distribution than the others reported here, and that therefore this root rot may be quite widespread in England and Scotland.

They also go to show that some soils appear to be heavily infected with this fungus because in several cases isolations have yielded it in great preponderance. It was isolated on many occasions at all seasons throughout the year, whereas *Coniothyrium Fuckelii* was isolated in greatest numbers in the early summer, while *Hainesia Lythri* was isolated mainly in the fall of the year. In our inoculation tests this fungus was noticeably slower in its action than the other fungi described.

Symptoms. Similar in all respects to those of the *Coniothyrium* root rot.

Etiology. Several strains of this fungus have been isolated; their spore characteristics are quite within the range for this species, but they show differences in colour and in macroscopic growth. These have not as yet all been tested for pathogenicity though the two or three strains that have been tested seem to suggest that they may differ in degree of parasitism. For instance, isolation No. 12 from East Malling produced in two weeks, with the preliminary method only, a brown discoloration of the inoculated roots, whereas an isolant from Scotland in the same time generally produced a "lesioned" or dead root. *Cylindrocarpon radicicola* Wr. has been tested fifteen times by the preliminary method and eleven times has given rotting of the inoculated roots. When tested by the oat hull method, somewhat inconsistent results have at times been obtained, though sufficient evidence has been secured to leave no doubt but that this fungus under the conditions of the experiments was capable of producing root rot.

In an experiment of five inoculated and three check plants set up last autumn, not a single case of definite root rot was obtained, but discoloration of roots occurred and a few incipient lesions were apparent. These plants were probably dug up for examination purposes too soon, as we have since ascertained that this fungus is a little slower in its action than those already discussed. The next experiment gave clear cut results as every one of the nine inoculated plants showed distinct lesions, while the check plants were healthy.

This year (1934) a small experiment of nine inoculated and six check plants was set up in February and to date (May 3rd) eight of the nine plants have approached the wilting stage, and many roots have been killed. The check plants are healthy. There is, therefore, good evidence for adding this fungus to the list as a parasite of strawberry roots under English conditions (Fig. 5C).

4. FUSARIUM ROOT ROT.

This root rot, caused by *Fusarium orthoceras,* is not only as widespread as the *Cylindrocarpon* root rot, but according to our tests, is much more destructive. In fact, our inoculation experiments to date place this root rot as being possibly the most important disease of the group studied.

Distribution. Fusarium orthoceras has been obtained from diseased roots from Kent (several different places), Sussex, Somerset, Wiltshire, Hampshire and Scotland.

Symptoms. The same as those described for *Coniothyrium* root rot except that the plants reached the wilted stage at about the time the plants inoculated with *Coniothyrium* were beginning to show definite stunting, but had not as yet begun to wilt.

Etiology. Fusarium orthoceras, like *Cylindrocarpon radicicola* Wr., was isolated at various times throughout the season. It was tested thirteen times by the preliminary method and proved to be strongly parasitic. In five days to a week, it was able to cause complete rotting of a fair sized root. Similarly, consistent and strong parasitic activity was demonstrated by the oat hull method. In all, some fifteen plants were inoculated with Fusarium and in every case the lesioned and rotted roots associated with root rot of strawberries were plentiful. In fact two months after inoculation the roots were so badly diseased that the plants wilted and died (Fig. 5A).

In an experiment carried out in the autumn of 1933 this root rot fungus produced definite lesions on roots which had been produced in inoculated sterilized soil by unrooted runner plants.

5. PACHYBASIUM ROOT ROT (*Pachybasium candidum* Sacc.).

This root rot disease was found on one occasion only, namely on badly diseased strawberries of the Madam Kooi variety in Surrey. This disease, therefore, may not be widespread and, generally speaking, may be of little importance, but it has been thought advisable to include it here because of the strong parasitic attack this fungus has made in our preliminary experiments.

Symptoms. Same as for *Coniothyrium* root rot, except that plants showed dwarfing and stunting followed by wilting earlier than the *Coniothyrium* root rot.

Etiology. The fungus responsible for this root rot has very kindly been identified provisionally for us by Mr. E. W. Mason, of the Imperial Mycological Institute, Kew, as *Pachybasium candidum* Sacc.

It has been tested three times by our preliminary method and three times by the oat hull method and strong root rot attack has resulted in each case. In fact, in an experiment set up in February for comparative results with *Coniothyrium, Hainesia, Cylindrocarpon, Fusarium* and *Pachybasium* root rots, it was noticeable that the three plants inoculated with *Pachybasium* showed the first symptoms of the disease and reached the wilted stage some days ahead of any of the other diseases. There seems to be very good reason, therefore, for considering that this fungus may be of importance in connection with general "root degeneration", in spite of the fact that the evidence to-day suggests it is very localized in distribution (Fig. 5B).

STRAWBERRY ROOT ROTS IN THE FIELD.

The above inoculation studies have demonstrated that there are at least five different fungi capable of parasitizing strawberry roots, and that therefore there are five distinct root rot diseases of the strawberry in England. The symptoms of these diseases in the field are identical. One test with *Cylindrocarpon radicicola* gave indications that under certain conditions, this fungus may produce more of a cortical rot than a complete root rot, and this possibility should be further explored, but in so far as diagnosis under field conditions is concerned, these diseases must be considered as a root rot complex until experimental tests have ascertained the organisms concerned. That is, root rot as it appears in a plantation may be a single disease, or any or all of the above diseases. From a field examination alone it is impossible to determine the causal fungus or fungi concerned. All that can be done is to diagnose for the presence or absence of root rots on the basis of symptoms.

Symptoms. A plantation affected with root rots presents an uneven and patchy appearance due to the small size of diseased plants and also because of the gaps caused by the death of severely affected plants (Fig. 1).

Plants affected with root rots appear dwarfed and stunted in comparison with healthy plants of the same age and variety (Fig. 2). The leaves and petioles of affected plants are greatly reduced in size, and often in number, but otherwise may appear normal, though the older leaves become brown in colour, and sometimes slightly yellow, particularly if growing in heavy, wet soil. The leaves do not show the marginal yellowing, puckering or distortion associated with virus diseases such as yellow edge (Fig. 9). However, as plants affected with root rot are often also infected with virus, insects or some other disease, it is sometimes most difficult to diagnose the respective symptom groups. Plants affected with root rot also present a flat appearance, due to the fact that the older leaves hug the ground, and the newer leaves, though erect, are small and comparatively close to the ground because of the shortened petioles. In cases where plants are severely attacked, the foliage wilts and this is particularly noticeable at fruiting time. If plants here and there throughout a plantation wilt at harvest time, the chances are that "root rot" is responsible. The explanation would seem to be that the extra water supply necessary to swell and mature the fruit is more than the depleted root system can provide, and wilting results.

Though the symptoms outlined above may suggest root rot, final diagnosis must depend on an examination of the root system. The roots of "root rot" plants show: (1) definite black lesions or cankers (Fig. 7) on the newer roots, (2) blackened and dead roots (Fig. 4), and (3) a noticeable lack of fibrous roots (Fig. 10). In making a diagnosis, only white or yellowish to brownish roots should be considered, since it is natural for the roots to be black in their second season. Therefore, comparatively new or present season roots are to be chosen for examination purposes. Such roots can be distinguished by their whitish to yellow brown colour, or by their position on the rhizome. In the strawberry new roots are for the most part produced above the older roots already formed. The roots nearest the crown are therefore the younger roots. If such roots are examined and they show (1) black lesions, (2) dead roots and (3) rotting of the cortical tissue (stele or core may be white), it is a case of root rot undoubtedly. On the other hand these newer formed roots on a healthy plant will show no lesions, dead roots, or black rotted cortex (Figs. 8A and 3A). It should be specially noted that root rot of the "black lesion" type first attacks the fine, fibrous roots, which are readily rotted away, then the cortex, and later penetrates into the core or stele. At any time on an affected plant it is therefore possible to find (1) a noticeable lack of fibre, (2) superficial cortical lesions, (3) complete penetration lesions, (4) cortical blackening of roots for some considerable distance (even entire length) and (5) roots completely rotted away. Where any or all of above symptoms are present, root rot is responsible.

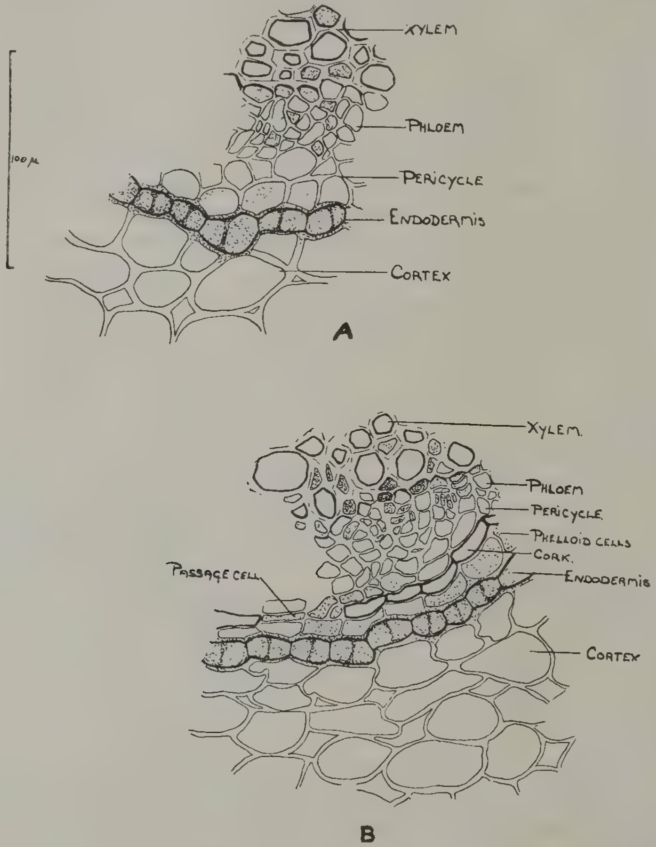
RUNNER PLANTS.

The roots of a healthy runner plant should go into the dormant season white or yellowish brown in colour (Figs. 3A and 8A). Therefore, if such plants show black lesions, or black cortical tissue, as is often the case, root rot is to be suspected. During the past season several severe cases of root rot on runner plants were observed.

Root rot may occur on a runner plant in any or all of its various forms, i.e. cortical rot, complete rot, lesions, and lack of fibre. The "cortical rot" was particularly prevalent in the clone planting cited above, and seemed to work mainly from the tip of the roots backward. The core or stele of many of these affected roots was as yet quite white and healthy.

ONE YEAR OLD AND OLDER PLANTS.

Diagnosis in such plants should be based on roots formed the fall before, which are therefore high up on the rhizome and are yellow brown to brown in colour, or on new roots formed in the current season and are therefore still higher on the rhizome and are white to yellow brown in colour depending upon the time of examination. All the large black roots at the bottom of the rhizome should be discarded in so far as diagnosis for root rot is concerned. If the above mentioned roots show conspicuous cortical rotting lesions or dead roots, and if


XYLEM
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XYLEM.
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CORK.
ENDODERMIS
PASSAGE CELL
CORTEX
B

FIG. 11.

A—Transverse section of a healthy portion of a root from a four-month-old strawberry plant showing no cork formation.

B—Transverse section of a diseased portion of the same root, nearer the tip, showing cork cells and collapsing cells of cortex.

fibrous roots are conspicuously absent, then there can be little doubt but that this is a case of fungous root rot.

The above symptoms of root rot are those associated with the disease under natural growing conditions where often more than one root rot organism is associated with the trouble.

THE LESIONED ROOTS.

It has already been pointed out that root rot symptoms may take the form of (1) lesions, (2) superficial cortical rot, or (3) dead roots. In connection with the superficial cortical type of injury a most interesting phenomenon has been noted, namely, a reaction to parasitic attack on the part of the strawberry by laying down a row or more of cortical cells in an endeavour to slough off the parasite present in the cortex.

It was particularly noted that the roots of runner plants from a virus-free clone, Royal Sovereign, runner bed showed an unusual amount of cortical rotting, often from the tip of the roots backward. The stele or core of most of these roots was white in colour and healthy. When such roots were sectioned and suitably stained it was noticed that cork cells had been formed, whereas no such cork cells were to be found in healthy roots of the same age and position on the plant. In many cases where this cortical rotting occurred in patches with healthy tissue in between, cork tissue was found only in the black or rotted areas and not in the white or healthy areas. This is clearly illustrated by taking a single root which had three distinct black or cortical rot areas on an otherwise healthy root. The different areas taken in order from above and examined showed (1) the black area nearest the crown with two layers of cork cells, (2) the white area below with no cork, (3) next a black area with a single row of cork cells, (4) a white area free from cork tissue, and (5) a black area extending to the tip. This seems to be a clear case of host reaction to parasitic attack. This root came from a two months old runner plant and therefore should not have had cork tissue, since normal sloughing of the cortex and cork deposition does not take place till the following season in such runner plants. Since cork tissue was present, as pointed out, it can only be explained as a host reaction to parasitic attack. According to Mann (12) a newly formed runner plant should not show decortication until the second season, because, he says, "During the period of flowering and fruiting there is pronounced browning and decortication of the older portions of the main adventitious roots. This is presumably due to the formation of a deep-seated cork layer for these blackened roots invariably show healthy central core etc." Again he writes, "The original 'runner' roots are yellow brown in colour when the first dormant season is reached." These quotations clearly point out that a normal runner plant should not have black roots or cork tissue when it goes into its first dormant season.

Etiology. Our isolation and inoculation tests have clearly demonstrated that root rot under natural conditions may be due to any or all of five different fungi. For instance, in one plantation at East Malling *Coniothyrium Fuckelii, Cylindrocarpon radicicola* and *Fusarium orthoceras* were the only parasitic fungi isolated from many plants, and therefore were most likely the only fungi responsible for the uneven condition of this particular plantation. Also, since all three fungi were isolated with equal ease it is likely that all three fungi were of equal importance in producing root rot. However, in one plantation *Cylindrocarpon radicicola* was isolated much more frequently than any other organism, while in another plantation the only parasitic organism isolated was *Pachybasium candidum,* and in a Sussex plantation only *Fusarium orthoceras* was isolated.

It should not be inferred from this that all these fungi display equal powers of parasitism against the strawberry root, because we already have some evidence that *Fusarium* and *Coniothyrium Fuckelii* are much more parasitic than *Cylindrocarpon radicicola.* For instance, it has happened on more than one occasion in a series of inoculation tests that *Fusarium orthoceras* and *Coniothyrium Fuckelii* produced root rot symptoms some days ahead of *Cylindrocarpon radicicola. Pachybasium candidum* in two inoculation tests also showed greater parasitism than *Cylindrocarpon radicicola.* It would appear from this that *Fusarium orthoceras* and *Coniothyrium Fuckelii* are the most virulent parasites of the five fungi, and that therefore they are the most important in connection with root rots of strawberry.

RESULT OF ROOT ROT ATTACK.

The ultimate result of root rot attack is death, though the time required to accomplish this end will depend on many factors such as soil temperature, moisture and cultural practices.

Though a plantation affected with root rot diseases may withstand attack sufficiently to produce two fairly satisfactory crops, often only a single crop of average poundage is obtained, and cases have been known where plantations planted in the autumn or spring were so patchy by next autumn as to warrant their being ploughed under. The progress of the disease is generally much slower than this in that it generally takes two to three years to affect a new plantation to such a degree as to make it unprofitable. A strawberry plant may be able to withstand the loss of a certain amount of its root system with no serious detrimental effects provided weather conditions are such that too great a strain is not placed upon the roots in their function of water absorbing organs. Therefore whether or not serious consequences result from the presence of these parasitic root fungi in the soil may largely depend on the suitability of any soil to act as a favourable culture medium for the fungi in question. If a soil is a suitable medium—and most soils appear to be—then sooner or later serious consequences must result. On the other hand if a soil is not a very favourable culture medium it is conceivable that the attack of the fungi under normal weather conditions may be so weak as to cause little, if any loss, but even in such a case, if conditions unfavourable to the strawberry plant occur, the reduced root system may result in serious consequences to the plant. A strawberry plant normally produces new roots and fibre in the spring and early summer and again after harvesting and well into the autumn. The two big peak periods of root production are in the spring and following harvesting. Therefore any fungus attack that is capable of destroying a large percentage of new roots shortly after their formation, must result in weak and sickly plants, if not in death. In those cases where new plantings have succumbed after a few months' growth the fungus attack must have been severe enough to destroy the new roots as formed. In fact, repeated examination of such plantations has shown this to have been the case. In this connection observational evidence suggests that the main root rot attack occurs from early spring up to and including harvest, but that after this time the attack is greatly reduced. From the above it is seen that root rot attack is a continual struggle between the plant and the fungi, the plant attempting to produce new roots to replace those destroyed by the root rot fungi.

The above may explain why it is that a plantation affected with root rot may last two or three years or, as is often the case, be so severely attacked the first year that the plantation is ploughed under as being unprofitable.

That severe destruction of roots results from the attacks of the parasitic fungi is clearly pointed out by an examination of the more recently formed roots on healthy plants and plants affected with root rots. In the case of healthy roots the younger roots—that is those that are white to yellow brown in colour—showed no lesions, and fibrous roots were plentiful. In the case of plants affected with root rots, fibrous roots were lacking, and the number of white to yellow brown roots was greatly reduced, and those that were present showed numerous conspicuous lesions. Counts made on three year old Royal Sovereign plants that, though affected with root rot, were still fairly thrifty, have given as high as 25 per cent. of the roots dead and 55 per cent. lesioned. In the case of plants more severely affected, the figures may be 50 per cent. dead and 50 per cent. lesioned. In fact it is a common thing to find not a single healthy root on severely affected plants.

These percentage figures give some idea of the "root pruning" caused by the various "root rot" fungi. If such root pruning were confined to a single period in the season its effect might not be serious, but our examination of roots throughout the growing season has led us to the belief that it is almost continuous, being influenced only by changing environment as to its rate and severity.

Though root rot can be very serious, indeed so much so as to render a plantation uneconomical after the first season's attack, this would seem to be rather the exception than the rule in England. On the other hand many plantations may be attacked with root rot for some months before any above ground signs are apparent. Even after they have been noticed the root pruning caused by the fungi may not be complete enough to cause serious loss provided the season is a favourable one for strawberry growth.

STRAWBERRY ROOT ROT AT EAST MALLING.

It might be well at this point to describe in some detail the progress of root rot diseases in two plantations at East Malling. When the senior author first inspected Newgates Field early in June 1933 the plantation appeared first-class in every respect; the stand was even, growth was good and the plantation on the whole appeared to be unusually healthy. Later in June, however, a few dwarfed and stunted plants were noticed here and there throughout the plantation, suggesting root rot, since the plants showed no virus or other disease symptoms. By the end of July, and before the plantation had been affected to any extent by mite attack, many plants showed the symptoms of ill health associated with root rot. Again in the autumn and the following spring "lesions" were commonly found on newly formed roots. This spring (1934) when growth started, the comparison between dwarfed and healthy plants was most striking. At the time of writing, May 23rd, six counts of six hundred plants taken in groups of one hundred plants in different parts of the plantation have given 42 per cent. of the plants definitely affected with root rot. Already the new roots formed this spring are showing lesions and the usual quantity of fibrous roots is lacking.

The second plantation to be described is a small runner bed of Royal Sovereign that consisted of eight maiden plants which produced about 1,800 runner plants. It should be specially noted that these plants were from a healthy, virus-free clone, and were kept free of mites, aphides and other insects by weekly spraying or dustings. Above ground these plants were therefore free of all leaf diseases and insects, and as they were from a virus-free clone there was no possibility of virus affecting them. Yet by the end of July many of the first formed runners showed signs of ill health and this condition increased until about 75 per cent. of the runner plants showed unmistakable signs of root rot. Examination of the roots showed the usual "lesioned" roots, dead roots and an unusual profusion of a type of cortical rotting. During the winter most of these runner plants were used for experimental purposes, but this spring five of the eight parent plants were dwarfed and unthrifty with typical root rot symptoms, and one of these has since died. This case is specially interesting in that all other possible factors—with exception of soil—such as leaf spot diseases, virus and insects were ruled out, and therefore the resulting damage was caused solely by root rot. On June 30th, 1933, several runner plants from this bed were struck in sterilized soil in pots while others were allowed to strike in the naturally infected soil outdoors. On November 7th, samples of both set of plants were dug up and examined when it was ascertained that the plants struck in sterilized soil had produced an extensive root system of healthy, white roots, while the plants—same clone and age—struck in contaminated soil produced a much reduced root system with "blackened" and "lesioned" roots (Fig. 8).

STRAWBERRY ROOT ROT AND YELLOW-EDGE.

Although Plakidas (6) found that Xanthosis did not affect the root system in its early stages at least, it seemed advisable to ascertain whether or not yellow-edge (Xanthosis ?) acted in a similar manner. Accordingly, runner plants from a healthy virus-free clone and runners from a yellow-edge clone were struck in sterilized soil on June 30th, 1933. At the end of the growing season on November 7th samples of these plants were examined when it was ascertained that the root system of both sets was perfect. This test, therefore, definitely points out that yellow-edge, in its first year at least, has no detrimental effect on the root system.

CONCLUSION.

The evidence recorded in this paper clearly demonstrates that strawberry root rot must be given serious consideration in connection with the present day degeneration of the strawberry in England. Experimental evidence has been given which proves that there are at least five soil fungi capable of parasitizing the roots of the strawberry causing ill health and often death. From the very nature of these diseases—a destruction of roots—it is obvious that they are of fundamental importance.

Degeneration of the strawberry in England has become increasingly apparent during recent years and various possible causes have been brought forward in explanation thereof, but none of these alone has fully explained the widespread degeneration.

Aphids, the Tarsonemid mite and yellow-edge have all been shown to be contributory causes, but where these have been controlled there still remains root rot as an important factor in strawberry degeneration.

CONTROL.

Though no investigations of a control nature have as yet been attempted, it is possible to make a few recommendations now that we have ascertained that root rots in England are caused by parasitic soil fungi. These recommendations are as follows:

(1) Only runner plants with white root systems should be used for planting purposes. Therefore carefully select runners, discarding all plants that show black roots. If "black root" plants are planted in new soil the root rot diseases are thereby added to the soil, and the diseases spread to the new plantation.

(2) Using runner plants with *white roots only*, these should be planted, if possible, on land that has not grown strawberries or raspberries, or if this is not possible, on land that has not grown raspberries or strawberries for at least four or five years.

Since some of the fungi associated with root rot of strawberries have also been found on raspberry roots, e.g. *Cylindrocarpon radicicola* and *Fusarium orthoceras* have been found on raspberry roots also, it is conceivable that ground which has carried diseased raspberries may be unsuitable for strawberries. It is considered inadvisable, therefore, that strawberries should immediately follow raspberries on the same ground.

ACKNOWLEDGMENTS.

The senior author wishes to acknowledge his indebtedness to the Director of the East Malling Research Station for the facilities placed at his disposal, and also for the keen interest shown throughout these investigations. The authors' best thanks are due to Dr. H. Wormald for his ready assistance at all times, for suggestions, and for his kindness in reading the manuscript, proof reading, etc., after the senior author returned to Canada.

SUMMARY.

The investigations reported herein are concerned with root rots of strawberry, of the "black lesion" type as found in Hampshire, Somerset, Sussex, Wiltshire, Dorset, Kent, Gloucester and Surrey, England. This type of root rot appears to be distinct from the "red-core" type as found in Lanarkshire, Scotland, and it is widely distributed in Europe and America. According to the literature "root rots" are considered to be of major importance in connection with the general deterioration of strawberry plantations.

A large number of isolations were made from diseased roots, and fungi of the following genera were isolated: *Coniothyrium, Hainesia, Cylindrocarpon, Fusarium, Pachybasium, Phoma, Botrytis, Chalaropsis, Rhizopus, Penicillium, Verticillium, Sphaeropsis, Cephalothecium, Rhizoctonia* (orchid type), *Pythium* and other Phycomycetes, and an unidentified sterile fungus. Nematodes were

found in the fine roots on a few occasions. Technique used in connection with isolations and with tests for pathogenicity is outlined.

As a result of inoculation studies, five fungi have been found to be capable of parasitizing strawberry roots, namely, *Coniothyrium Fuckelii* Sacc., *Hainesia Lythri* (Desm.) v. Höhn., *Cylindrocarpon radicicola* Wr., *Fusarium orthoceras* App. and Wr., and *Pachybasium candidum* Sacc. Accordingly five root rot diseases are recorded for the strawberry, though the symptoms of all five are very much alike, if not identical. Under field conditions any one or all of these diseases may be present in a plantation. A healthy virus-free, insect-free clone of Royal Sovereign was used in connection with all inoculation tests and therefore the possibility of "virus" or "insects" aiding the root rot fungi has been eliminated.

The symptoms for all five diseases comprise dwarfing and stunting of leaves and petioles and browning and drying of lower leaves, resulting in a flat type of plant. Plants severely attacked wilt and die. The roots of affected plants show (1) lack of fibre, (2) distinct black lesions on roots (white to yellow brown in colour), and (3) dead roots. The roots of "root rotted" plants are noticeably deficient in fibre.

The nature and sequence of attack appears to be associated primarily against the finer and younger roots. In the spring the first symptoms are apparent on roots formed the previous autumn, followed later by attack against roots formed in the current season. A reaction on the part of the host plant to "throw off" the fungus is indicated by the laying down of cork cells in affected areas.

The detrimental effect of root rots is illustrated by describing in detail the result of root rot attack in two plantations at East Malling.

A comparison of runner plants from a healthy clone, and a yellow-edge clone of Royal Sovereign showed that the roots of both sets of plants were equally healthy. Therefore yellow-edge in its first season at least has no detrimental effect on the root system.

It is contended that the root rots described herein play a major rôle in the general degeneration of the strawberry because of the almost constant "root pruning" to which attacked plants are subject.

Control measures at the present time consist in (1) selection of runner plants free from "black roots" and (2) crop rotation. A resistant variety offers the best means of control for a disease of this kind.

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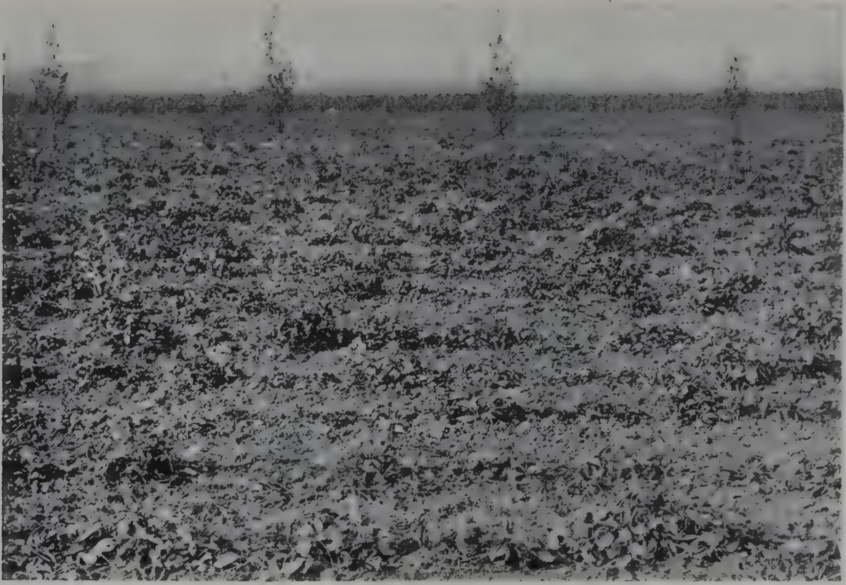


FIG. 1.

Plantation at East Malling affected with root rots. Note the patchy condition of the plantation.

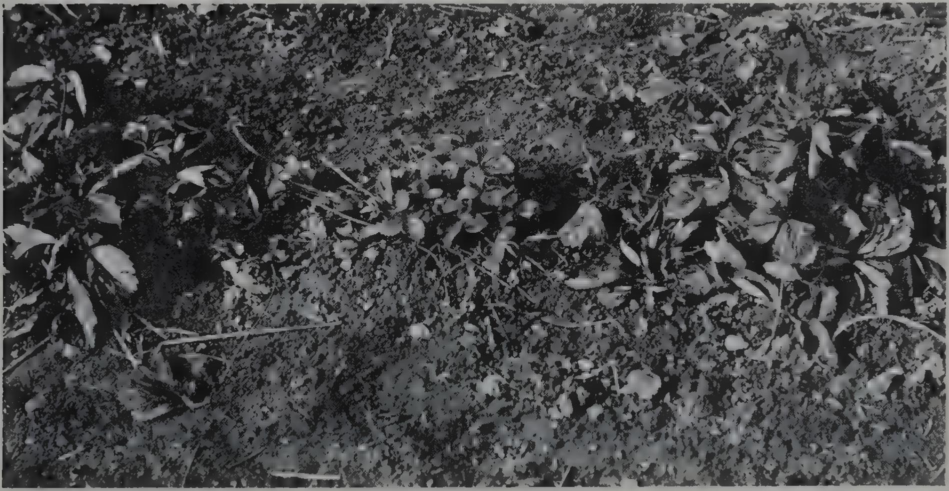
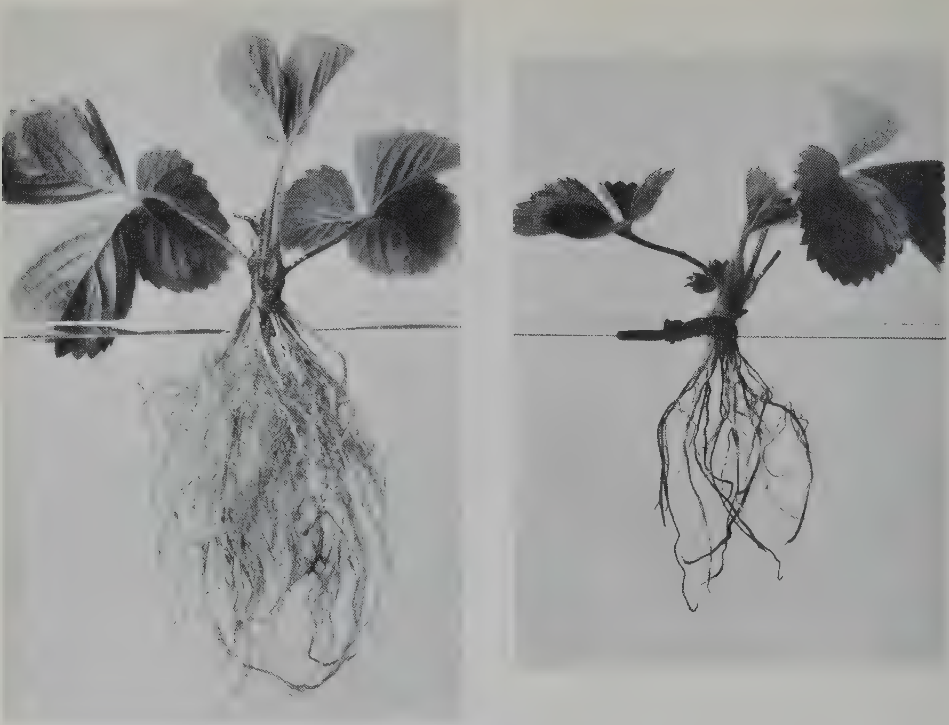


FIG. 2.

Root rot plant in centre. Note its dwarfed condition in comparison with fairly healthy plant on right.



A FIG. 3. B

Root Maceration Inoculations. A—Control plant. B—Inoculated plant.
Note reduced root system and black rotted roots in B.

FIG. 4.

Root rot on runner plant. Note lesions and dead roots (black).

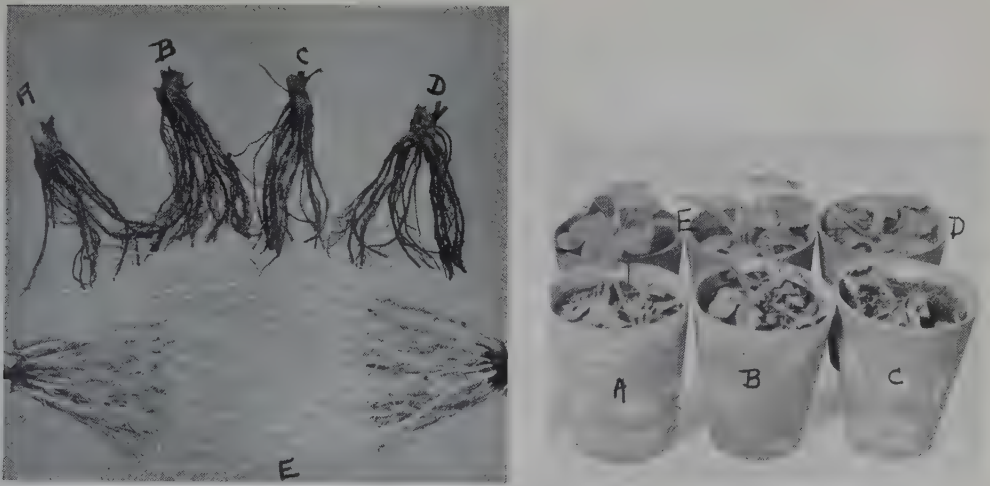


Fig. 5.

Inoculations with pure cultures of A—*Fusarium orthoceras*. B—*Pachybasium candidum*. C—*Cylindrocarpon radicicola* and D—*Coniothyrium Fuckelii*. E—Control.
Note dead roots of inoculated plants and vigorous white healthy roots of control.

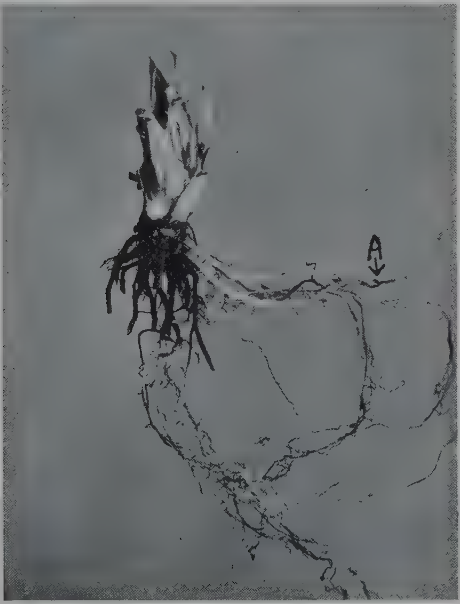


Fig. 6.

Plant inoculated with *Coniothyrium Fuckelii* in October 1933.
Examined and found as above in April 1934.
Note roots in centre actually rotted away. Note lesion at A.

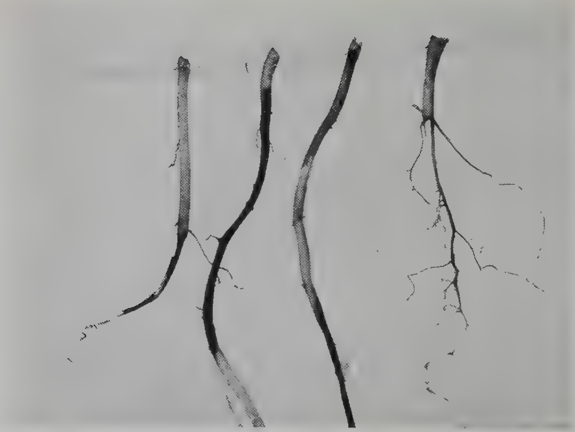


FIG. 7.

Typical black lesions on naturally infected roots.

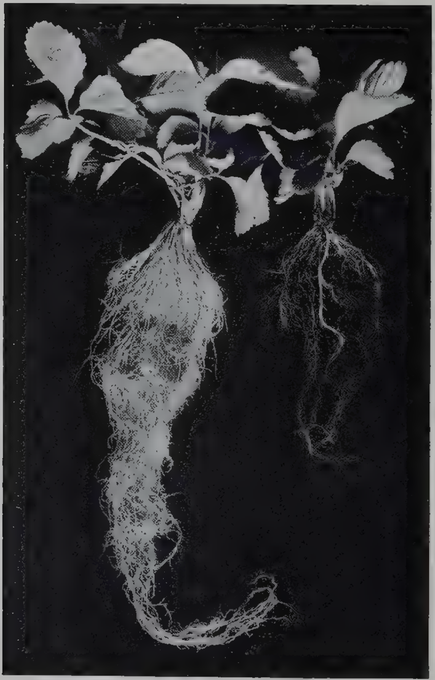


FIG. 8.

A—Control grown in sterilized soil. B—Natural infection from clone runner bed. Plants of same age and parentage.

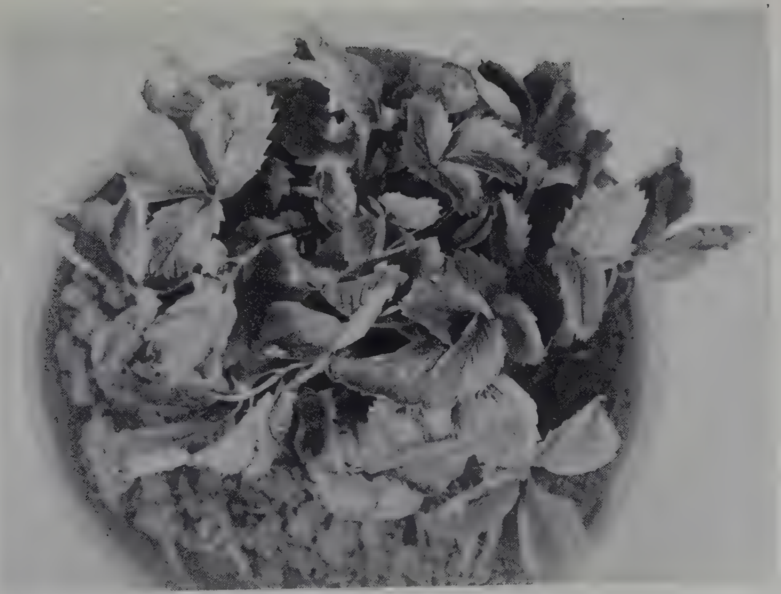


Fig. 9.
Enlarged view of root rot plant seen in centre of Fig. 2.

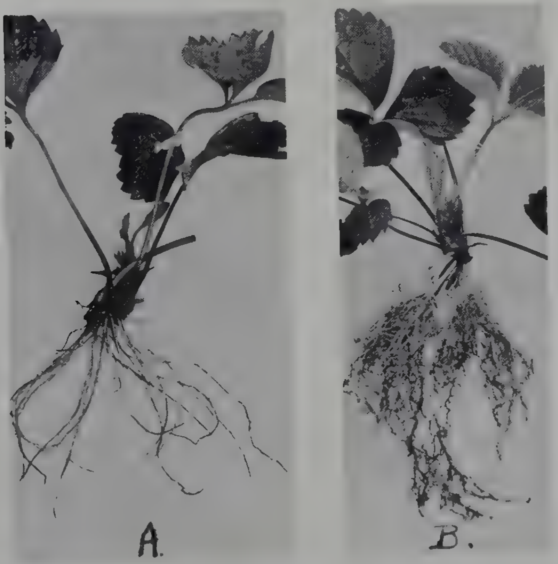


Fig. 10.
A—Runner plant from runner bed severely affected with root rot. Note lack of fibre. B—Runner from runner bed with trace of root rot. Note profusion of fibrous roots.

THE JONES-BATEMAN CUP FOR RESEARCH IN FRUIT-GROWING

In 1920 Miss L. Jones-Bateman of Cae Glass, Abergele, presented to the Royal Horticultural Society a valuable silver-gilt replica of the Warwick Vase to be used for the encouragement of fruit production. It is accordingly decided to offer it triennially for researches in the growing of hardy fruits, figs, grapes and peaches in the open or under glass, and it is available for award in 1934.

Candidates should submit accounts of their work by October 31st. The work dealt with must have been carried out by the candidate in the United Kingdom mainly during the past five years. The Cup will be held for three years by the successful candidate who must give a bond for its safe return, and when the Cup is relinquished the holder will receive a commemorative gold medal. The holder will be eligible to compete on the next or any succeeding occasion.

The Assessors will be three, two appointed by the Royal Horticultural Society and one by the National Farmers' Union, and they will report to the Council of the Royal Horticultural Society upon the originality and comparative potential value to the fruit growing industry of the work of the candidates.

The Council of the Royal Horticultural Society will award or withhold the Cup at its discretion.

F. R. Durham, *Secretary*.

BOOK REVIEWS

VIRUS DISEASES OF PLANTS. By John Grainger, Ph.D., B.Sc. pp. viii. + 104. Oxford University Press, 1934. 6s.

The great interest that has been shown in virus diseases of plants during recent years is indicated by the number of published articles, dealing with various aspects of these diseases. This interest is not merely academic, for many of the diseases seriously threaten the productivity of some of our most useful crop plants, and the economic side has rightly received great attention. Much " fundamental " work, with no immediate practical bearing, is being carried on, however, for there is much to be learnt regarding the nature and properties of viruses, their mode of infection and their transmission, and the solving of these problems should lead ultimately to more effective measures of control.

That the intensive study of virus diseases has received an impetus in recent years is shown by the list of " References " in Dr. Grainger's book. There are 445 of these references (they are stated to be " representative, not exhaustive ") and about 400 of them are to papers published since 1920.

The study of virus diseases cannot be neglected by anyone who is interested in the growing plant, for virology is now an extremely important section of plant pathology. The author of *Virus Diseases of Plants* states in the preface that " the aim of this book has been to introduce the student to the phenomena associated with Virus Diseases ". It is a small book, at a low price, but the attempt to get much information into little space prevents it from being little more than a notebook. The student, however, will find it useful for reference, particularly in the sections describing laboratory technique and general experimental work. The physiology, properties, and transmission of plant virus, and the classification of virus diseases are dealt with ; one chapter describes " The Relation of Insects to Virus Diseases ".

Chapter V contains an interesting account of the methods that have evolved for controlling virus diseases, and discusses among others such measures as " rogueing ", the use of resistant and immune varieties, vegetative propagation of virus-free stocks, and " Indexing " (as applied to potatoes). Apart from this the book will probably make no great appeal to the practical horticulturist, for in the chapter devoted to specific diseases only eight are described, and but one of these concerns fruit—Raspberry Mosaic, which is dismissed in twelve lines.

One important feature of these investigations is the testing of viruses by means of inoculation experiments, and the technique of this is outlined in the last chapter. One important method of artificial transmission has been omitted here, that of " patch-grafting ", by means of which a patch of bark, taken from a diseased plant, is grafted on a healthy plant. The method was used with success by Sreenivasaya a few years ago (Ref. No. 382 in Dr. Grainger's book) in his investigations on the Spike Disease of Sandal, and more recently by R. V. Harris (unpublished work) in his Raspberry Mosaic studies.

The book is illustrated by six plates from photographs, and five text figures.

H.W.

THE " DEGENERATION OF THE STRAWBERRY ". Imperial Bureau of Fruit Production, Technical Communication No. 5 (price 2s., The Imperial Bureau of Fruit Production, East Malling, Kent).

Of the many horticultural problems that have vexed both grower and research worker within recent years, none has proved more puzzling or more difficult to solve than that designated the " degeneration of the strawberry " in the title of the above mentioned publication. This complicated matter has exercised the minds of pomologists, chemists, plant pathologists and entomologists, and each has contributed something of value to the common stock of knowledge ; but no one can claim that the exact cause (or causes) of the trouble has yet been conclusively demonstrated, much less that a complete remedy has been found. In these circumstances both research and advisory (extension) workers will be grateful to the Bureau for presenting a survey of the position as seen from the point of view of a pomologist (Mr. Akenhead), a worker in virus diseases (Mr. Harris), a mycologist (Dr. Berkeley), and a zoologist (Mr. Massee). Each writer has dealt with his aspect of the subject with commendable moderation, presenting impartially the results of both British workers and those overseas, whilst particularly welcome to those studying the problem will be the full list of literature references given at the end of each chapter. There is no concluding section to sum up the results of the discussion ; indeed, any such summary would seem to be premature, hence the reader is left entirely free to form his own conclusions. Perhaps the most that can be gathered in this respect is that the writers tend towards the conviction that the main difficulty in growing good crops of strawberries to-day is due to disease (using the term in a wide sense) rather than to cultural or manurial defects or to a hypothetical " physiological degeneration " of the strawberry plant itself. Professor Barker, who provides an interesting introduction, points out that, however serious may have been the effect of the bad culture to which strawberries were subjected during and immediately after the war, there has now been ample time for recovery, and he evidently would look elsewhere for an explanation of the problem. In this connection, however, one point might perhaps have been further elucidated : the reader is left with the impression that the present yield from an average strawberry plantation is but a small fraction of that normally produced before the war ; and this view would certainly be supported by growers, who would also point to the relatively short life of most modern plantations. But it must not be forgotten that it is human nature, when looking back, to remember the " good " old times, forgetting those that were not so good ; and it would perhaps have contributed to a better balanced view of the situation if some statistical data showing the extent to which strawberry yields have shown reduction during the past twenty years could have been brought forward. For instance, in connection with the Census of Production Act, 1906, an enquiry into the acreage and yields of certain crops was completed for the year 1908. It showed that the estimated average yield from 28,815 acres of strawberries was no more than 29 cwt. to the acre. For comparison it may be added that the average yield in 1931 was some 30 cwt. per acre. Even allowing for the fact that 1908 was an unfavourable year for strawberries and 1931 a good year, it would seem possible that the degree of so-called " degeneration " of the strawberry may have been overestimated.

Finally, since the Bulletin will doubtless soon require revision, a minor criticism—or perhaps, rather, suggestion—may be offered in respect of the title. On the evidence available, indeed on the evidence in the publication itself, the term " degeneration " is not too apt ; moreover, it conveys to the layman, apparently, a meaning much more sensational than the position actually warrants. It is clear that even if the retrogression is mainly due to pathological conditions it cannot yet be ascribed to the attacks of any one single pest or disease, but may result from a number of interacting factors. Hence a title such as " The Causes leading to unsatisfactory crops of Strawberries " would seem to be preferable to one that may lead to sensational and premature conclusions.

J.C.F.F.

THE SEASONAL CYCLES OF NITROGENOUS AND CARBOHYDRATE MATERIALS IN FRUIT TREES

2. THE SEASONAL CYCLES OF ALCOHOL SOLUBLE MATERIALS AND OF CARBOHYDRATE FRACTIONS AND LIGNIN IN THE WOOD, BARK AND LEAVES PORTIONS OF TERMINAL SHOOTS OF APPLE TREES UNDER TWO CULTURAL SYSTEMS—GRASS PLUS ANNUAL SPRING NITRATE AND ARABLE WITHOUT NITROGENOUS FERTILIZER

By **ELSIE S. SMYTH, B.Sc., Ph.D.**
Long Ashton Research Station

INTRODUCTION.

The investigations concerning carbohydrate materials described in the present paper are to be regarded as complementary to those reported for nitrogen by Karmarkar in Part I of this series. The two investigations were carried out simultaneously on samples taken from the same trees and on the same occasions, with a view to obtaining data relating to nitrogen and carbohydrate cycles which could be utilized to study the inter-relationships between these two important classes of materials over the annual growth cycles of the trees.

In the introduction to Part I, Karmarkar has outlined the scope of the whole series of proposed investigations and, in stressing the importance of studies on nitrogen cycles has indicated the desirability of considering such cycles in relation to the parallel cycles for carbohydrate materials ; hence it is unnecessary to discuss nitrogen investigations further at the present stage. The value attached to studies of carbohydrate metabolism by workers in problems of fruit tree nutrition will be evident from the following section dealing with the results of previous work in this field.

The results herein reported relate to cycles of alcohol soluble materials, reducing sugars, sucrose, starch, hemicellulose, cellulose, and lignin in the bark, wood and leaves of terminal shoots.

They show clearly that these substances exhibit definite seasonal cycles associated with growth phases in all three portions of the shoots (this being especially evident for sugars and starch), and serve to throw light on the uses of the various constituents in connection with the phenomena of translocation, storage reserves and structural functions. The effects of the two nutritional treatments given to the trees on the cycles and levels of these constituents, are shown to be of only minor importance.

SURVEY OF PREVIOUS WORK.

A considerable amount of work has been carried out since the close of the nineteenth century on the seasonal distribution of various carbohydrate substances in plant tissues. Deciduous trees, evergreens and herbaceous plants have all been included in the investigations, and under the first heading, orchard trees naturally occupy a prominent position.

The chief problems for investigators working along these lines have been briefly, the determination of the relationship between the carbohydrate content and the growth responses of the plant to various treatments (such as manurial stimulus or ringing) and the elucidation of the mechanism of translocation and storage.

It has been recognized for a long time that the degree of vegetative growth and fruitfulness is largely dependent on carbohydrate supply and that the leaf area (and therefore the potential capacity for photosynthesis) is a relative index of the amount of dry matter formed. Other factors such as nitrogen and moisture are of similar importance and since the work of Kraus and Kraybill (21) in 1917, most chemical studies have been concerned with the relation between carbohydrate and nitrogen content, for these workers had shown that variations of this ratio are associated with different physiological states of the plant with regard to the amount of vegetative growth and fruitfulness.

The examination of seasonal cycles is important in any work of a comparative nature, such as the present, in which the effect of two methods of cultivation (arable and grass + spring dressing of nitrate) is under consideration: this also applies to most of the problems studied by earlier workers.

In the investigation reported here, terminal shoots only were examined and the following historical review will be limited to work relating to these structures and to spurs. The crop from the trees analysed was practically negligible that year so that no information was obtained with regard to the composition of the fruit.

Several investigators have studied the seasonal changes in the spurs from the standpoint of fruit bud formation and the conditions affecting it, for example, Hooker (17), Mitra (25), Harvey and Murneek (15), Potter *et al.* (30, 31). In their work the spur was analysed as a unit, no separation into bark and wood being practicable. Later work has shown that in analysis of shoots, this separation is necessary if any seasonal series of estimations is to be made, owing to changes in the proportion of bark to wood; and of course such a division gives a much clearer understanding of the functions of the tissues concerned. Traub (44) recently has divided the shoot into four layers, a further step in this direction, but experimental difficulties and error are liable to increase with the number of tissues separated.

Most of the earlier work on shoots was also carried out without this separation of tissues, Fischer (10), Le Clerc du Sablon (35), Butler, Smith and Curry (2), Mitra (25), Harvey (13) and Roberts (34) all having analysed the whole twig. It is not until 1925 that investigations on bark and wood began to appear, by Proebsting (33), Thomas (42), Traub (44) and finally Kraybill and Sullivan (22) in 1930. Swarbrick (39, 40), in 1927 and 1928, studied changes in both tissues microchemically. Some valuable information was acquired in the work with the "whole twig" method and the results obtained in this way are thus included in the short review presented here.

The soluble carbohydrates examined in previous studies include reducing sugars, sucrose and total sugars, and also maltose, although the major portion of this substance, found by Mitra (25), has since been shown to be Phloridzin by Harvey (14). Starch was also estimated by various methods, some of which, such as acid hydrolysis, obviously include substances other than starch. Starch and sugars are naturally of great interest on account of their known close connection with photosynthesis, and it is from the seasonal fluctuations of these substances that the more valuable information has been obtained.

Hydrolysis of plant material with mineral acids (usually 2 per cent.) after extraction of sugars, yields a fraction containing alcohol insoluble polysaccharides, including starch. If the value obtained for starch by enzyme action be subtracted, the remaining fraction is generally termed "hemicellulose", consisting of complex polysaccharides which on hydrolysis break down into sugars such as mannose, galactose and pentose. Gehardt (12a) in 1929 for one year apple wood, Widdowson (48) in 1932 for fruit and Tottingham (43) in 1933 for twigs have obtained figures for various constituents of this fraction. The occurrence and changes in amount of the hemicelluloses have been studied by Nightingale (27) for salvia and tomato, and by Murneek (26) and Proebsting (33) for apple shoots; opinions differ as to its function as a reserve food or structural substance, which is not surprising considering the varied nature of its constituents.

The final product of hydrolysis by strong sulphuric acid (80 per cent.) "cellulose", has been estimated in apple shoots by very few people, Roberts (34) being one example. Seasonal analyses of this fraction have been made in the present work.

The results of the investigations mentioned (whether working on spurs, whole shoots, or separated tissues), are further discussed below in relation to certain carbohydrate fractions.

BARK AND WOOD.

Sugars. The importance of sugars for respiration, translocation and the synthesis of more complex substances makes it obvious that some form of seasonal cycle of these must occur. Observations, whether on spurs or shoots, show that a curve with a single maximum in the winter and a minimum in spring, is the general rule, the chief increase in sugars taking place after September. Hooker (17), working on spurs, and Mitra (25), on spurs as well as one and two year twigs, obtained similar results. Hooker, however, found that the date of the minimum differed according to the sterility or productivity of the spur. Harvey and Murneek (15) observed that considerable individuality existed within the tree itself, so that a spur was largely dependent on its own leaves for food supply. In Harvey's work (13) on the growth of summer shoots, he also noted that the tip, middle, and basal portions of a twig contained different amounts of food material, and that substances which tend to decrease during the growing season are always most abundant in the tip (water, soluble solids, phloridzin and nitrogen), while those that normally increase are most abundant in the base (insoluble solids, sugars, polysaccharides and total carbohydrates).

Thomas (42) worked with one year shoots, grown under different manurial conditions, particularly in respect to nitrogen, and found that glucose and sucrose decrease rapidly during active growth and later accumulate. His results showed that trees receiving nitrogenous manure contained higher concentrations of simple sugars than those without nitrogen.

Traub (44) obtained the usual October accumulation of sugars in bark and wood of two-three year twigs, with the spring minimum and approximately constant content for wood over the growing season, but in the bark a rise from the spring minimum to a higher level in June.

Kraybill and Sullivan (22) in their summary referred only to sucrose, and found that it increased in wood but decreased in bark from November to December, while the reverse occurred from February to April. They do not consider sucrose to be a primary reserve carbohydrate, and state that it is always found in relatively small quantities throughout the tree. In the present work the sucrose value was always lower than the reducing sugar figure, but the content appeared to undergo definite seasonal changes and to show relatively large increase in autumn. Phillis and Mason (29) suggest sucrose as the chief sugar of translocation in the cotton plant, from the regularity of the fluctuations observed.

Starch. The seasonal changes of starch content have been the subject of much discussion, owing to the fact that a cycle showing two maxima and minima has been noted by several investigators (Fischer (10), Le Clerc du Sablon (35), Cameron (3), Mitra (25), Kraybill and Sullivan (22)), while the microchemical observation of Price (32) and Swarbrick (39) also afford confirmatory evidence.

The first minimum occurs in the young shoot at the time of maximum leaf growth. This is followed by gradual starch accumulation and a starch maximum during leaf fall with a subsequent fall to a winter minimum and a second maximum in very early spring. This double maximum curve was also found for starch in spurs by Hooker (17). It is interesting to note that Cameron (3) working in a mild climate at Davis, California, found a winter minimum similar to that of plants growing in regions where there are severe winters. He also examined seasonal changes in young orange trees (4) in 1932, but found only a single maximum for these evergreens.

Swarbrick (39), 1927-8, studied starch changes in tissues of one-five year old branches. He noted the disappearance from cortex and phloem in January and early February, reappearance at the end of February (first maximum), and complete disappearance from all tissues of one-four year branches in May (first minimum), this occurring basipetally. Starch accumulated at the end of the elongation period, beginning in the apical regions of the shoots, leading to a second maximum content.

Kraybill and Sullivan (22), 1930, found "rather large quantities" of starch in all parts of the tree (Stayman apple) even during periods of maximum utilization of reserves ; this is at variance with the complete disappearance noted by Swarbrick (39). Kraybill and Sullivan confirm the presence of the two maxima in November and April, with minima in December and May. Further discussion on the winter minimum has been postponed to the section dealing with starch estimations.

A few other points of interest in connection with starch content may be mentioned. Roberts (34), analysing whole twigs, confirmed Harvey's work on regional distribution of starch in the twig, but also found that the starch content generally appeared to be inversely proportional to the nitrogen content, trees with much nitrogen exhausting the starch reserve by about blossoming time, and only restoring it late in the season, while those without much available nitrogen do not exhaust the starch supply except close to the growing points. Fruit production delayed starch storage, and the latter was closely related to cessation of terminal growth.

Thomas (42) found that the starch cycle in wood lagged behind that of the bark. He obtained the usual autumnal accumulation and noted that his work appeared to confirm Hooker's (19) suggestion that storage of starch seems to be the cause of growth retardation rather than the result of it. Carbohydrate accumulation was promoted by deficiency of nitrogen in his experiments, but he found that lack of phosphorus or potassium could produce similar effects.

Polysaccharides other than Starch. The seasonal changes of the hemicelluloses (i.e. polysaccharides distinct from starch), have been estimated by Thomas (42), Murneek (26), Kraybill and Sullivan (22), Proebsting (33), Hooker (17) and Harvey (13) among others, while Traub (44) sub-divided this group determining the pentosans, and by subtraction of this figure from the total acid hydrolysable fraction left a residue of "acid hydrolysable hexosan" which, however, included starch.

As previously mentioned, opinions differ with regard to the functions of this fraction, owing to the varied nature of its components. It is obvious that a decision on this point must be difficult, and the suggestion of Nightingale (27) that it has a twofold function, partly structural and partly as reserve food, seems the most acceptable view. Some of the results of the investigations mentioned above are of interest in this connection.

Proebsting (33) observed no significant fluctuations from April to September in this fraction, for bark or wood of one year apple shoots—he did not work through autumn and winter—and that even after defoliation, with consequent deficiency of starch, hemicellulose was not used as a reserve. Thomas (42) also found the fluctuations relatively small in one year bark and wood, and concluded that hemicelluloses are not indices of immediate seasonal changes, as they are not very labile, but that they function chiefly as reserves. Kraybill and Sullivan (22) assign "a less important" place as reserves to hemicellulose, than to starch and sugar, owing to the indefinite character of the fluctuations. Hooker (17) (for spurs) noted an increase in spring until May, which in bearing spurs, continues to a high maximum in September with subsequent decrease, while in non-bearing spurs during May and June the content decreases, and then rises again about the middle of the fruit bud differentiation period. Barren spurs gave cycles differing with the variety, but all three types accumulate to a September maximum and then decrease. The above thus offers evidence of a regular seasonal change.

Murneek (26) supports the view of the importance of hemicellulose as a reserve food, from results on apple spurs and reproductive organs analysed at short intervals during the summer. He also supports Schellenberg's (36) observations on the reciprocal nature of the starch-hemicellulose content in plants. This relation appears to occur also in Traub's (44) work where increase in pentosans is accompanied by relative diminution in the hexosans.

Cellulose and Lignin. Very little work appears to have been done on the estimation of these fractions in apple wood, or any investigations of seasonal changes, and no data on lignin analyses have been found in the literature here reviewed. These constituents are obviously structural in function, and their determination in the present work was undertaken with a view to obtaining as complete a picture as possible of the carbon containing fractions in the tissue. The results are discussed later. Roberts (34) gives some figures for cellulose and notes that the observed changes in content give an index of the time when the cell walls change in composition and begin to be lignified or altered to hemicellulose.

LEAVES.

Here again there are very few data on the carbohydrate changes in apple leaves throughout the season. Harvey (13) analysed leaves for total carbohydrate for June, July and August and obtained an approximately constant content. Durant, quoted by Chandler (6), however, studied the seasonal variations in compound carbohydrates and simple sugars. He found two periods, first one of synthesis and polymerization, during which all carbohydrates increased until the end of the summer, and secondly one of hydrolysis and simplification with the onset of leaf tinting; during this period more complex carbohydrates decrease but simple sugars accumulate. The recent photosynthetic experiments of Heinicke (16) in 1932 on ringing effects on apple leaves *in situ*, are not, of course, directly comparable, but his method may be of interest in later work in this connection; it involves estimation of the carbon dioxide rather than the synthetic products. Phillis and Mason (29), 1933, have carried out detailed estimations of the sugars and polyglucosides in cotton leaf extracts, from the point of view of translocation problems, obtaining figures for diurnal variation and the effect of ringing.

The work of Murneek and Logan (26a), 1933, on the changes in nitrogen and carbohydrate content of leaves during leaf fall (October and early November) should also be mentioned here.

It is thus apparent that very little is known of the seasonal composition of apple leaves over long periods and an attempt has been made in this investigation to determine sugars, starch, and hemicellulose in leaves obtained from the shoots collected for analysis.

CARBOHYDRATE / NITROGEN RELATION.

The association of the plant with certain equilibria between the concentrations of carbohydrate and nitrogen, with regard to development, either in vegetative growth or fruitfulness, has been generally accepted, the most important evidence being the work of Kraus and Kraybill, previously mentioned. Later investigators have all made some attempt to consider this relation when studying the effects produced by the particular treatment of the plants in their own experiments. Among these, Hooker (18), Harvey (13), Roberts (34), Nightingale (27) on Salvia, Proebsting (33), Traub (44) and Potter and Phillips

(30), may be mentioned. The work of these writers on the carbohydrates has already been discussed. The available results on nitrogen content have been reviewed by D. V. Karmarkar (20a). An important conclusion reached in regard to nitrogen is that not only total nitrogen, but its fractions, such as protein and soluble nitrogen constituents should be considered separately, as some of them appear to be very closely connected with growth responses. This point is discussed at the end of this paper where the results of the nitrogen analyses made by Karmarkar are compared with the carbohydrate figures obtained from the same trees. Potter and Phillips (30) conclude from their studies on C/N ratios that the relationship cannot be given any mathematical form, nor can any particular value obtained be given as a constant figure for one set of conditions.

EXPERIMENTAL.

MATERIALS AND SAMPLING.

The material for analysis was obtained from the variety Newton Wonder grafted on Malling No. II stock, planted in 1923; the differential nitrate treatment was started in 1929, and the samples were collected from April 1932 to March 1933. A description of the condition of the plots and method of sampling has been given in the paper on nitrogen analyses by Karmarkar (20a). Each sample for the carbohydrate investigation consisted of 50-60 shoots of the current year's growth.

The material was preserved in 85 per cent. alcohol (allowance being made for moisture content of the shoots) as soon as possible after sampling, to prevent further changes in the nature of the carbohydrates. The prepared material was dropped into hot alcohol which was then brought to the boil and allowed to continue boiling for 1-2 minutes after which the bottle was stoppered while hot with a waxed cork. Calcium carbonate was only added for the alcoholic extraction in the soxhlets, as some previous experiments showed no difference between the results for material preserved for a few weeks in alcohol and those in alcohol + calcium carbonate. Bark and wood samples consisted of about 60 gm. fresh weight and leaf samples of 100 gm. Duplicates were taken of every sample.

DISCUSSION OF CHEMICAL METHODS.

Estimations were made of the following constituents:

1. Alcohol soluble matter.
2. Reducing sugars and sucrose.
3. Starch.
4. "Hemicellulose."
5. Cellulose } for bark and wood only.
6. Lignin-containing residue }

DISCUSSION OF METHODS.

Determination of Sugars. Shaffer and Hartman's (37) "Macro" method (cuprous titration) was selected for these estimations as the sugar concentrations were too low to be determined by a method such as that of Lane and Eynon. The reduced copper is estimated by means of thio-sulphate; this was satisfactory for wood, but the cleared bark extract was found to be yellow coloured and this affected the end point of the iodine reaction. The reduced copper oxide was therefore filtered off and estimated by the Permanganate method of Bertrand. For the sake of uniformity the wood extracts were also treated in this way. Gardner (11) has employed a similar type of modification for pear tissues.

The iodometric determination was therefore used only for glucose derived from starch, hemicellulose and cellulose, where the material had already been alcohol extracted and there was no colour difficulty. The error on pure glucose was approximately 1 per cent., calculating glucose from copper by Shaffer's graph. It was found later, however, that this copper if determined by permanganate gave too high a result with Shaffer's graph. The permanganate was therefore standardized directly against A.R. glucose (0-100 mg.) and the graph obtained (a straight line) employed for converting the copper values to glucose. The factor 0·95 was used to convert glucose to sucrose. Total sugars are represented as total glucose present after citric acid hydrolysis.

Starch. The methods for starch determination are all open to some criticism. Digestion with Taka diastase has been employed by Davis and Daish (8) combined with polarimetric determination of the mixture of glucose and maltose produced. This has been criticized by Horton (20), who found Taka diastase unreliable in its effect. Bish (1) on bracken, estimated the reducing power of the glucose only and related it to a starch scale of copper equivalents; this has been criticized by Widdowson (37), 1932, because it assumes the constancy of the glucose/maltose ratio, which she found to be variable. She determines the two sugars separately by iodate and ferricyanide methods, which appear to be suitable for the range of variation in the quantities of starch found in apple fruits. Gardner (11), 1929, using saliva, related his sugar estimations after digestion to a scale obtained from estimations of the sugar after enzyme action followed by acid hydrolysis.*

Shriner (38), 1932, employed a formula based on several assumptions, including the constant ratio, to convert the glucose value after Taka diastase digestion, to starch; the writer obtained high results on applying this formula to figures from Shaffer and Hartman titrations after use of Taka diastase. Proebsting (33) used Taka diastase followed by acid hydrolysis, in which some sugar may be destroyed during the acid treatment, as noted by Widdowson, and also some other polysaccharides in the extract hydrolysed. Thomas (41) digested with Taka diastase; he stated that the ratio of sugars was constant, and estimated the glucose with picric acid.

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* Gardner's paper was not read until after the writer's method had been employed for some time, but his method would appear to give results approximating more closely to the true starch value.

Other investigators have employed either saliva, e.g. Otterson and Tottingham (28), Harvey (13), Murneek and Logan (26a), or malt diastase, e.g. Walton and Coe (46), followed by acid hydrolysis. Nightingale (27) hydrolysed with acid after Taka diastase digestion. Tottingham subtracts a pentose equivalent from his "crude starch" value, but this method is tentative as yet.

The method employed here was that of digestion by malt diastase followed by acid hydrolysis with 2 per cent. hydrochloric acid in a boiling water bath for two and a half hours. Extraction of the starch was found to be incomplete after grinding the tissues to pass 100 mesh sieve and boiling the aqueous mixture for half an hour before digestion. Autoclaving at 120° C. for two hours as suggested by Proebsting (33) finally proved successful.

This method is open to the two criticisms of Proebsting's method given above. Davis (7) found slight destruction on boiling glucose with acid for some hours. Mason and Maskell (24) however found that fructose but not glucose was destroyed, the latter resisting the action of 0·6 N (approximately 2½ per cent.) hydrochloric acid at boiling point for three hours. The results of the experiment given below confirm this. A glucose solution, treated as in the above described starch method, was neutralized after boiling with acid and estimated by Shaffer's method.

Controls.				After 2½ hours heating.	
No acid.		Acid in cold.			
Glucose.		Glucose.		Glucose.	
Taken.	Found.	Taken.	Found.	Taken.	Found.
0·5008	0·4985	0·5006	0·5044	0·5008	0·5044
0·5008	0·4985	0·5006	0·5074	0·5010	0·5032

Estimations on bark alone, and on bark + added starch (B.D.H. potato starch purified by water, alcohol, and ether washings) gave the following results, with (1) 4 gm. air dry bark alone, 2 sets, and (2) 2 gm. bark + 0·2 gm. starch (dried at 70°), 3 sets.

4 gm. Bark alone.	Starch + 2 gm. Bark.	Added Starch.	
		Found (B–A).	Taken.
0·4002 0·3962 } 0·3964 ∴ 2 gm. 0·1982 = A	0·3951 0·3926 } = B 0·3951	0·1969 0·1944 } 0·1961 0·1969	0·2 gm. – moisture at 100° C. (2·26%) = 0·195

The results show that starch can be satisfactorily estimated in the presence of the plant tissue. Widdowson (47) states that starch dried at 100° C. still contains 3·6 per cent. moisture, a point which has not been investigated, but the above results would then be 4 per cent. too high. That this is a constant error, however, was found by similar results being obtained with pure starch alone, potato or soluble.

The error on the starch determination on bark was found from five sets of the same ground sample (each of 4 gm.) and is calculated as the standard error of the mean:

Starch weight in sample.	As % air-dry weight.
0·4320	11·70
0·4449	12·06
0·4346	11·78
0·4398	11·91
0·4297	11·43
M=0·4362±0·0009	M=11·78±0·106

The second criticism of the method is due to the fact that other hydrolysable polysaccharides occur in the filtered starch extract, as Otterson and Tottingham (28) have shown. Removal of these by the alcohol precipitation of Walton and Coe (for large amounts of polysaccharides in linseed) was not satisfactory, the method being tedious and employing large quantities of alcohol, besides which the polysaccharides, if present, were so dilute that no visible precipitate was obtained. It is also known that alcohol does not always precipitate pectin in dilute solutions. Pectin estimations (Nightingale (27a), Carré and Haynes (5)) in the autoclaved extract gave, however, only 0·5 per cent. dry weight soluble pectin for wood and no precipitate with bark or leaves. (Total pectin by acid extraction = approximately 15 per cent. bark and leaves, 1 per cent. wood.) Pectin is, therefore, not a source of error here. Tottingham, however, as previously mentioned, finds some pentosans which, by the acid hydrolysis method employed here are included in the "starch" estimation, but their determination would have been impracticable for the present work.

"Hemicellulose." This fraction has been variously estimated: (1) as the loss in weight after acid extraction of the starch free materials, e.g. Tottingham (43), who, however, has also analysed this fraction; (2) by the reducing power (× 0·9) of the products of acid hydrolysis, e.g. Nightingale (27); and (3) by the difference between the weight of the alcohol insoluble material, and starch + pectin (as calcium pectate) + residue insoluble in N/75 hydrochloric acid, e.g. Widdowson (48).

The loss in weight after acid extraction was observed to be greater than the hemicellulose figure estimated from reducing power. This was found also to occur in these experiments, and is accounted for by Tottingham's analysis of the extract which showed that this fraction contains varying amounts of crude protein, uronic anhydride, pentosans and glucosans.

In the present experiments Waksman and Stephens' (45) hydrolysis by 2 per cent. hydrochloric acid, at boiling point for five hours, was carried out on the ground material after alcoholic extraction of sugars. Subtraction of the "starch" value gives a figure for polysaccharides other than starch and this was termed hemicellulose. For wood this figure is roughly 20 per cent. dry weight, and for bark 10 per cent. The loss in weight during extraction however (minus the hemicellulose figures above) is still about 15 per cent. in wood and 20 per cent. in bark; this has not been included under any definite fraction, but shows only slight variations throughout the year.

As the acid extraction is carried out by boiling the mixture for five and a half hours, some sugar will be destroyed, as the figures for one experiment on glucose show below, and if the starch figure previously obtained is subtracted from the total polysaccharides it will leave the hemicellulose figure below its true value; this is again an unavoidable but small and constant error. Murneek (26) has studied this hemicellulose fraction, however, and considers that its estimation is of considerable value in the interpretation of seasonal changes in the plant reserves.

Results of experiment on boiling solutions of glucose in 2 per cent. HCl for five hours (as for hemicelluloses).

Taken gm.	Solution in cold HCl.	After 5 hours boiling.	Loss.
0·25	(1) 0·2531	(1) 0·2431	0·0064
	(2) 0·2512 } 0·252	(2) 0·2438 } 0·2432	=2·4%
		(3) 0·2431	

Cellulose. Treatment of the residue from the hemicellulose extraction with concentrated (80 per cent.) sulphuric acid, the method adopted by Waksman and Stephens (45), has been employed. The above workers obtained recovery of 95 per cent. pure cellulose, and preferred the sulphuric acid treatment to all other hydrolysis methods. No experiments on pure cellulose were made by the writer, the above method being followed in detail.

Lignin containing residue. The residue from the above extractions consists chiefly of lignin, with some impurities. On igniting this residue the ash left was found to be negligible, the mineral matter and nitrogen having been extracted during the previous operations. The ignitable fraction thus consists very largely of lignin and has been termed "crude lignin".

DESCRIPTION OF CHEMICAL METHODS USED.

These are given in detail in the Appendix.

EXPERIMENTAL RESULTS.

All the results have been calculated in terms of the percentage dry weight of the respective plant material, bark, wood and leaves. Determinations of lignin and cellulose were not made on the leaves. The "dry weight" was calculated from the weight of the residue after alcohol extraction, and the weight of alcohol soluble matter in the extract.

In Tables I and II of the complementary paper on Nitrogen by D. V. Karmarkar (20a) data are given with regard to the collection of samples, condition of the shoots, moisture content, and percentage of bark and shoots, etc. so that repetition of these details is unnecessary here.

The results are presented graphically as follows:

1. Seasonal cycles of the individual constituents for bark and wood (Figs. 1-11) and leaves (Figs. 12-18).
2. Summary of all the cycles plotted to the same scale, for bark, wood and leaves of grass plot (Fig. 19).
3. Carbohydrate / total nitrogen and carbohydrate / total non-protein nitrogen ratios (Figs. 20, 21).

Note.—In all the graphs the value obtained in April for shoots nearly a year old has been connected by a line to that for the young shoots in June, there being no May sample. These connecting lines are not, however, intended to represent the carbohydrate content in shoots during the period April to June.

DISCUSSION OF RESULTS.

SEASONAL CYCLES OF INDIVIDUAL CONSTITUENTS.

In the following discussion the results are considered with reference to the physiological periods of plant growth as described by Karmarkar in discussing the nitrogen data obtained in the complementary investigation. These periods are as follows:

Period of bud swelling—April and May.

Shoot elongation—June, July and part of August.

Full growth—late August, September and most of October.

Leaf fall—late October and November.

Dormancy—December, January, February and March.

It will be found that the curves representing the cycles for grass and arable plots are almost always similar in form so that any description of the general trend of a cycle refers to both plots. It must be noted that no samples were obtained for March or May 1932.

FIG 1.

TOTAL ALCOHOL SOLUBLE MATTER

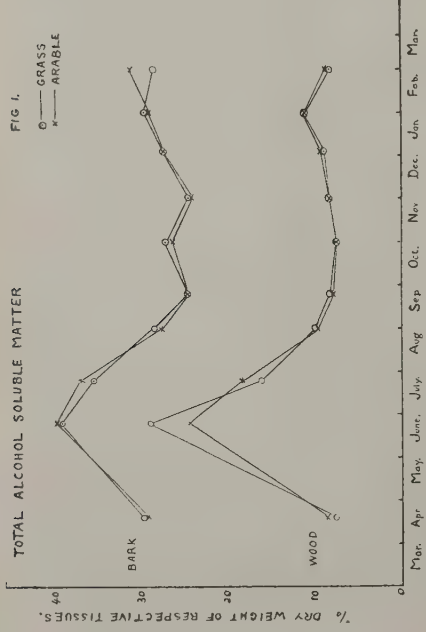


FIG 2.

ALCOHOL SOLUBLE MATTER MINUS SUGARS

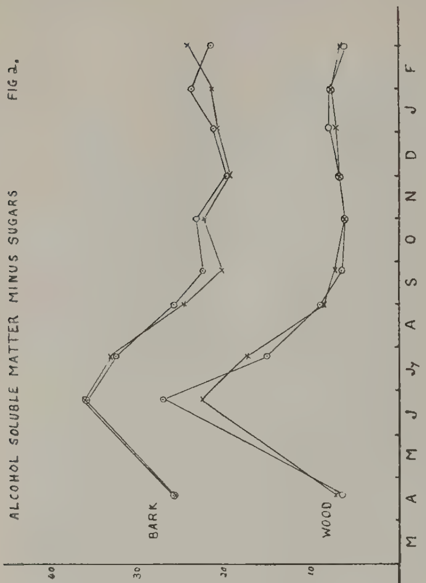


FIG 3

REDUCING SUGARS

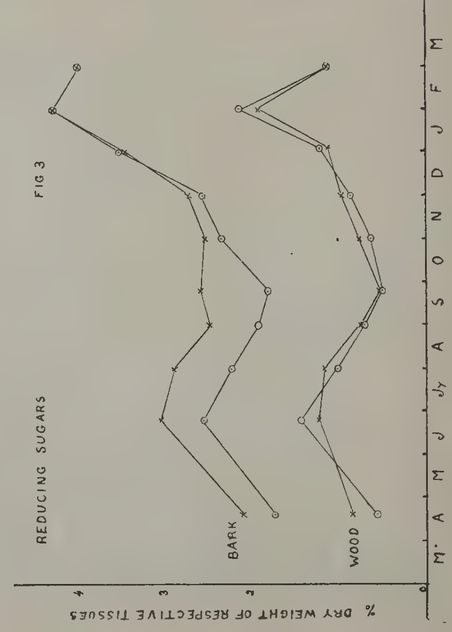
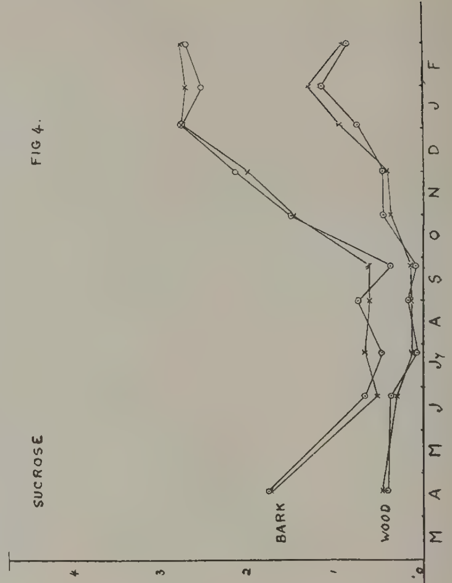


FIG 4.

SUCROSE



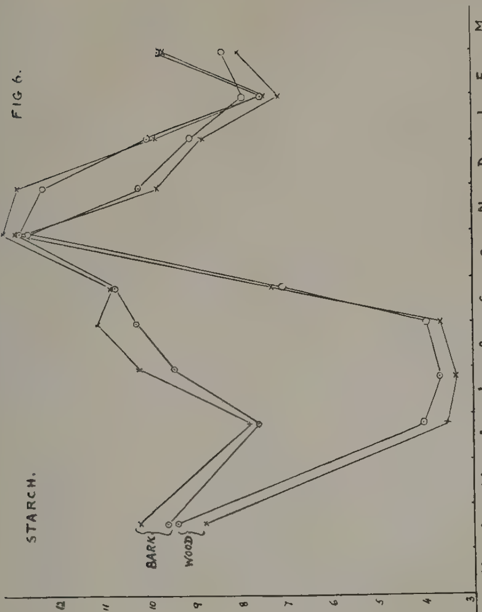


FIG 6.

STARCH.

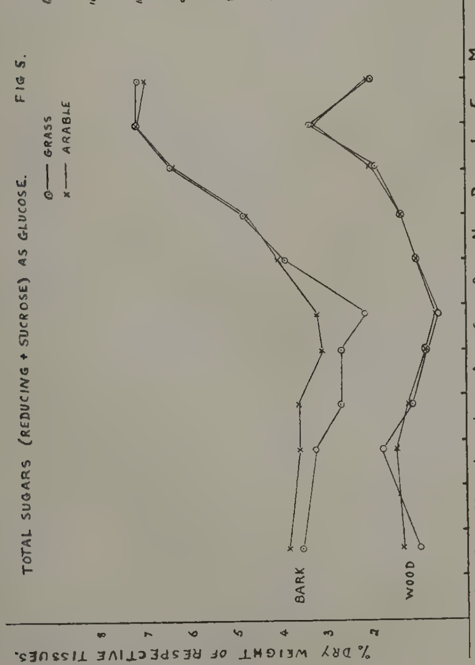


FIG 5.

TOTAL SUGARS (REDUCING + SUCROSE) AS GLUCOSE.

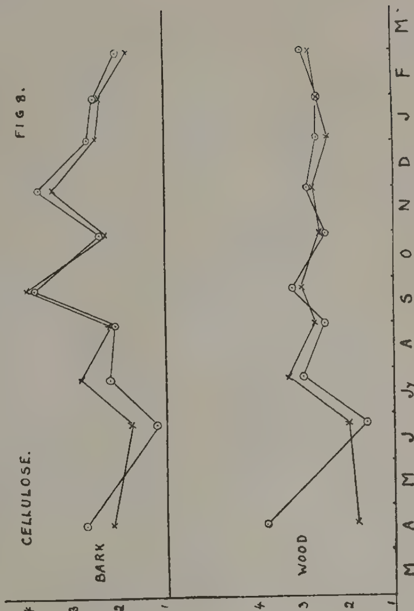


FIG 8.

CELLULOSE.

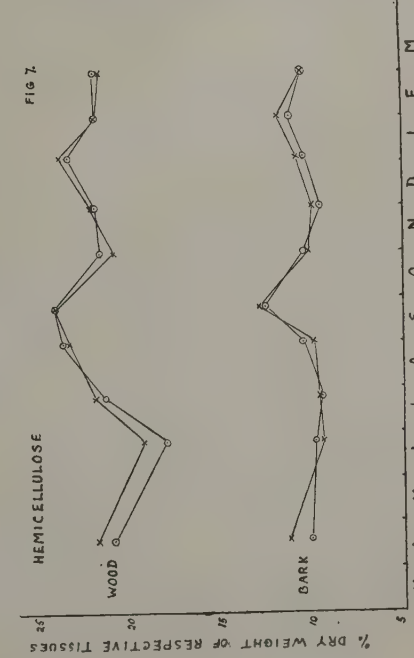


FIG 7.

HEMICELLULOSE

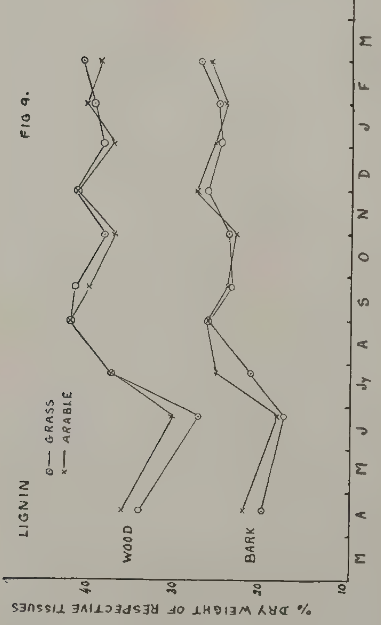


FIG 9.

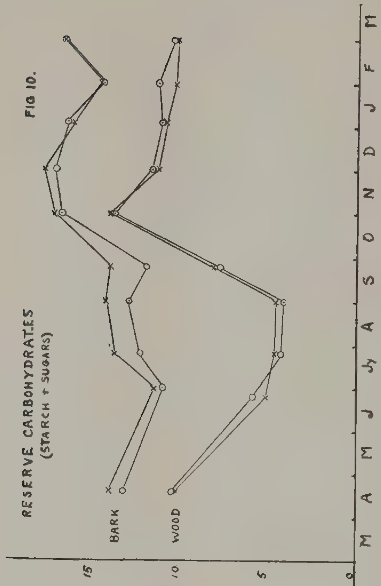


FIG 10.

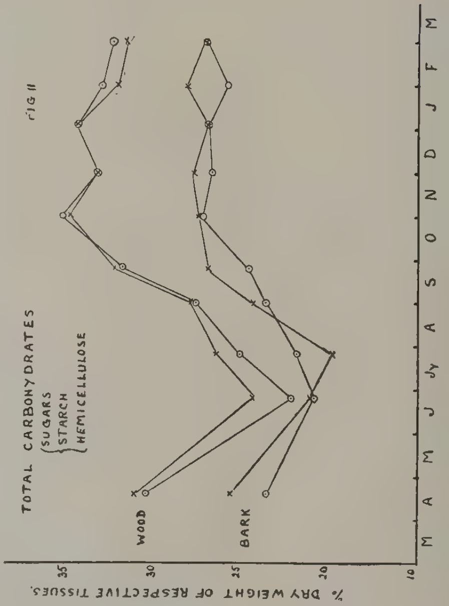
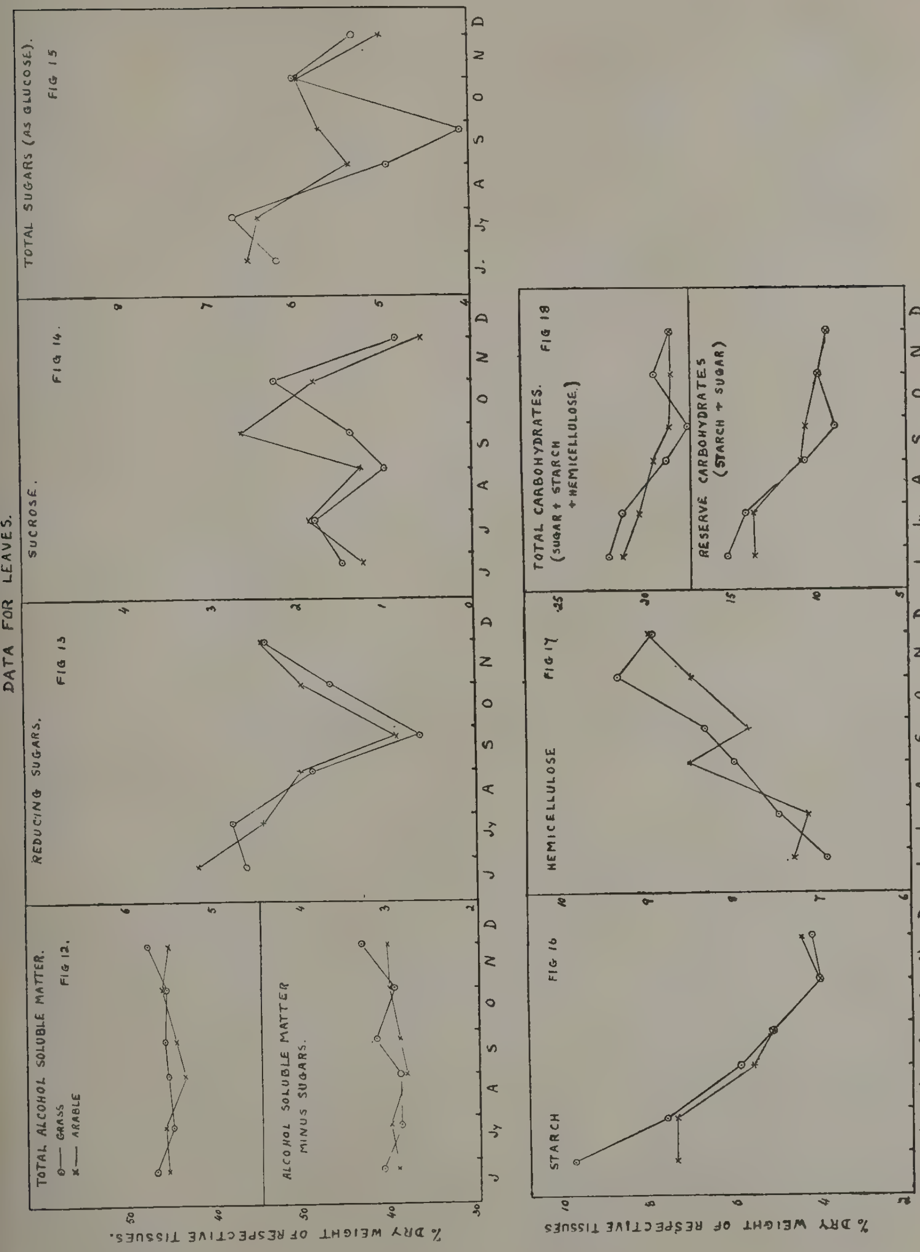


FIG 11


DATA FOR LEAVES.
TOTAL ALCOHOL SOLUBLE MATTER.
FIG 12.
o— GRASS
x— ARABLE
ALCOHOL SOLUBLE MATTER MINUS SUGARS.
REDUCING SUGARS.
FIG 13
SUCROSE.
FIG 14.
TOTAL SUGARS (AS GLUCOSE).
FIG 15
STARCH
FIG 16
HEMICELLULOSE
FIG 17
TOTAL CARBOHYDRATES. (SUGAR + STARCH + HEMICELLULOSE.)
FIG 18
RESERVE CARBOHYDRATES (STARCH + SUGAR)
J Jy A S O N D
% DRY WEIGHT OF RESPECTIVE TISSUES.
50
40
30
6
5
4
3
2
1
0
8
7
10
9
25
20
15

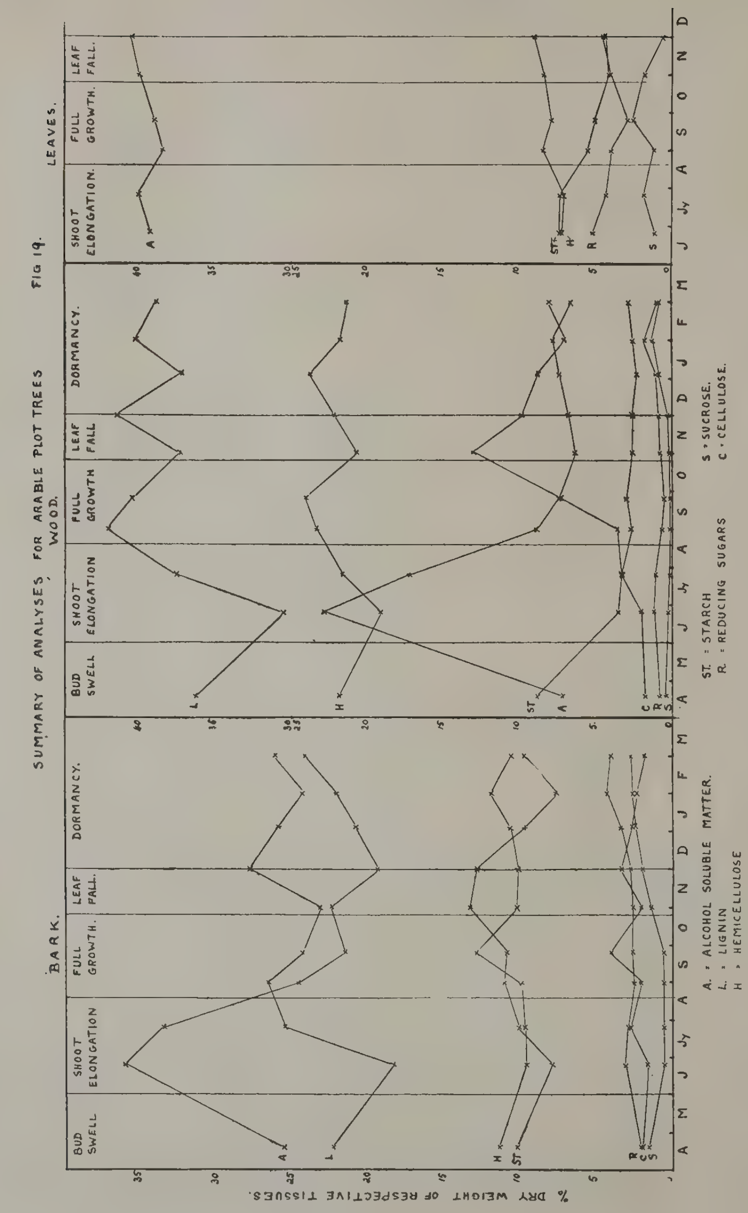


FIG 19.

SUMMARY OF ANALYSES FOR ARABLE PLOT TREES

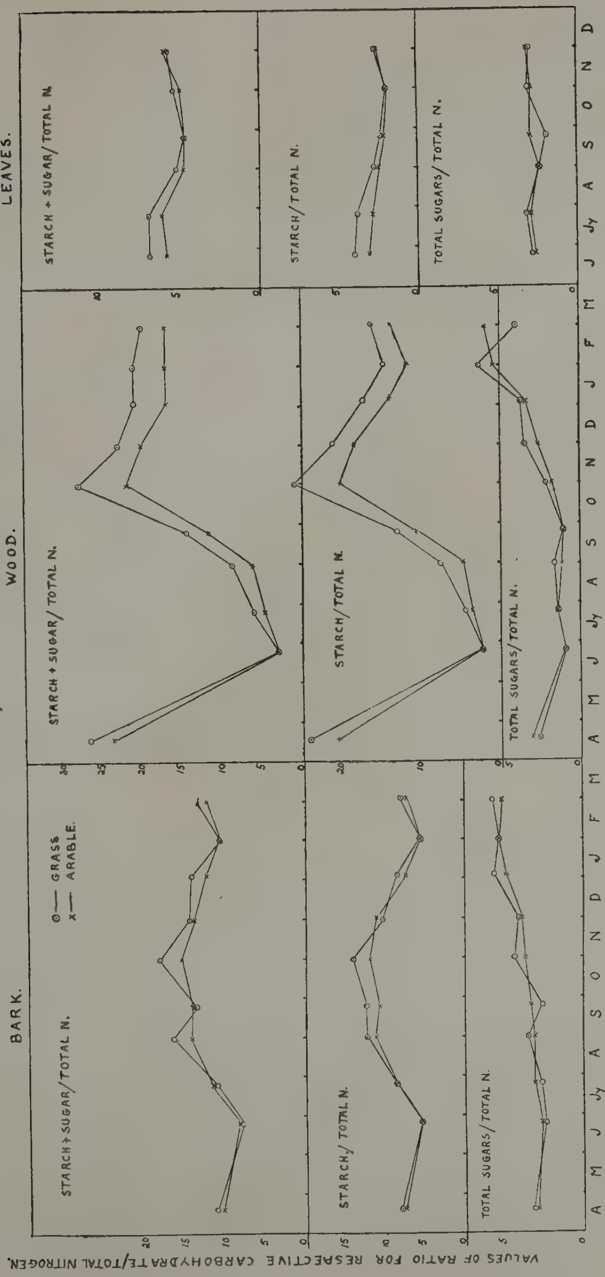
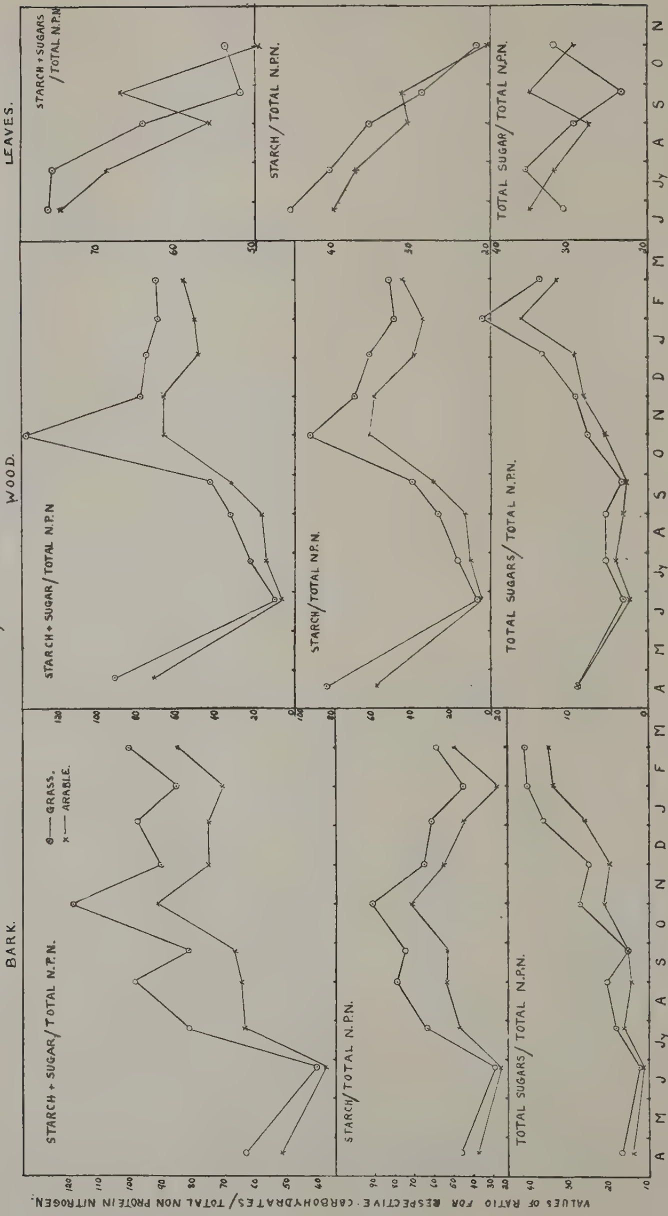


FIG 20

CARBOHYDRATE/TOTAL NITROGEN.

FIG. 21.

CARBOHYDRATE/TOTAL NON PROTEIN NITROGEN.



BARK AND WOOD.

Total Alcohol Soluble Matter (Fig. 1). This fraction, which includes sugars, colouring matter, phloridzin, and a variety of unidentified substances, shows very similar cycles for bark and wood. There is a maximum value in June, the first sample of new shoot growth, which is preceded by a low value in the one year old shoot in April, and followed by a decrease throughout the growing season, until the end of September for bark and October for wood. The bark then shows a temporary rise (8 per cent. of the previous value) in October, decreasing again in November. Both tissues now show slightly increasing contents during the dormant season.

The concentration in the bark is naturally much higher than in the wood, and ranges from a maximum of 40 per cent. to a minimum of 24·5 per cent., while in the wood the figures are 29 per cent. and 7·5 per cent. It can be seen from these figures that the rise in the wood in June is much greater than in the bark and this difference persists when curves are plotted for the alcohol soluble matter after subtraction of the sugar values (Fig. 2).

Comparing these figures with those obtained by Karmarkar* (20a) on the same samples, for water soluble matter, it will be found that the curves for wood in the two cases are almost identical in form, the actual values for water soluble materials being 3-5 per cent. lower than the alcohol values, except during the leaf fall and dormant season when the difference is only 1 or 2 per cent.

In the curves for *bark* the similarity is again observed, and includes the gradual rise during the dormant season noted in the alcohol soluble graph and also the October peak. The difference in concentration is greater than for the wood, that is, the total water soluble matter is generally about 7 per cent. lower throughout.

It is thus obvious that the young shoot has a very high relative percentage of substances soluble in water and alcohol, which are subsequently used during the growth period. The slight increase in the bark during the dormant season, over that observed in the wood, appears to be due to increase in sucrose, which is also greater in bark than wood. This is confirmed by the lower gradient of rise in the curve for alcohol soluble matter minus sugar.

The maximum content of soluble matter in June which also coincides with the maximum water content in the tissues is, of course, the condition one would expect to find in a young, actively growing shoot, and has been observed by other investigators, notably Harvey (13), who divided the shoot into tip, middle and base; he found that these soluble substances were most abundant in the tip, the decrease during the growing season, however, taking place along the whole shoot. He also noted that phloridzin (which is included in alcohol soluble matter and had a maximum concentration in the tips of 10-15 per cent. dry weight) is most abundant in early summer, falling off throughout the growing season. Phloridzin would therefore account for a considerable proportion of the alcohol soluble matter in the June maximum observed in this work.

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* It should be noted that Karmarkar's curves include values for May 1932.

There is no significant difference between the grass and arable plots either in the nature of the cycle or the actual figures.

Reducing Sugars (Fig. 3). The cycles for bark and wood are again very similar, the values for bark being consistently higher than those for wood (the former ranging from 4·31-1·74 per cent. and the latter 2·15-0·15 per cent.).

The young shoots in June show a peak value, which decreases steadily till the end of September; then follows a gradual increase to a much higher second maximum at the beginning of February, in the middle of the dormant period. During the next month the content falls more rapidly in wood than bark. A very low figure occurs in April (of the previous year) for one year shoots, which thus indicates that there is a continuous decline from February until April (or May), after which accumulation of sugar takes place while the buds are swelling.

The first decrease during the growing season is obviously due to the use of sugar in growth and respiration, and the increase during the periods of full growth and leaf fall is due to translocation from the leaves. The sugar content in the bark is consistently higher in spring and summer for arable plot trees than grass. This condition may be correlated with the fact that the arable plot trees were rather more vigorous, as previously mentioned. The decrease after the winter maximum is chiefly due to the synthesis of starch which occurs prior to the spring bud swelling, and will be discussed later in connection with the starch figures.

Traub's (44), 1927, results for cortex phloem in two-three year shoots are very similar to those obtained in this work for bark. He finds a rise from May to a June peak, then an almost constant period until a further rise in September to a maximum about December. A winter maximum occurred also for wood, but no June peak.

Sucrose (Fig. 4). The sucrose values for bark and wood are consistently lower than the corresponding reducing sugar figures, varying in bark from 2·79-0·49 per cent. dry weight, and in wood 1·3-0·08 per cent. The curves show only one maximum, in winter, at the same point as that for reducing sugars. Thus both sugars accumulate during the latter part of the summer and early winter, but between April and June the sucrose decreases while reducing sugar increases so that in June when the young shoots, now about seven inches long, show a peak value for reducing sugars, the sucrose is almost at a minimum. In the bark the reducing sugars increase by 0·97 per cent. dry weight and 0·83 per cent. (arable and grass) while the sucrose decreases by approximately

1·21 and 1·09 per cent. dry weight. In the wood reducing sugars increase by 0·4 and 0·9 per cent. (arable and grass plots) while the sucrose only falls 0·2 and 0·04 per cent. The sucrose decrease in the wood is thus insufficient to account for the rise in reducing sugars which, however, may also result from hydrolysis of starch present there, or the translocation of sugar from bark and leaves (which were full grown in the June sample). Phillis and Mason (29), 1933, consider that sucrose is used as the medium of sugar translocation, from detailed investigations of its fluctuations in the leaves and stems of the cotton plant; with apple shoots, as far as these results go, sucrose appears to be more of a temporary or primary storage product. The fact that its concentration is low, and even decreasing at the main translocation period of bud swelling (as is the case for starch) and remains low for the rest of the growth period, would support this view, as also does the fact that its value is only appreciable during periods of carbohydrate storage. The curves also suggest sucrose as an alternative storage product to starch during the coldest period of the dormant season, starch again appearing in spring before active translocation is required.

Kraybill and Sullivan (22) noted an increase in sucrose during early spring in bark, but a decrease in wood, and a decrease from November to December in bark with corresponding increase in wood. There is no evidence of similar changes from the results presented here, as sucrose increases steadily through the autumn in both tissues. The fact that both sugars and also starch increase in the autumn may be regarded as due to translocation from the leaves and is discussed further below.

Grass and arable plot figures are again not significantly different and have therefore not been discussed in detail.

Starch (Fig. 5). The cycles for both tissues show two maxima and two minima, and differ only in the depth and duration of the summer minimum. This point is referred to again in the discussion of hemicellulose content.

At the beginning of the growth period (June) a minimum starch content is found (8 per cent. dry weight bark, 4 per cent. wood). In July and August the starch increased to approximately 10·5 per cent. in the bark while it remains at a minimum in the wood. In September and October, however, both tissues accumulate starch, the wood more rapidly than the bark, so that by the end of October a maximum of 13 per cent. has been reached, bark and wood at this point having the same percentage content. Then follows a steep decline in the content of both tissues until, by the end of January, approximately the same percentage content is reached at the second and winter minimum (7·5 per cent.). There is thus no complete disappearance of starch at this period. The starch begins to accumulate again from this point until the end of February, the last sample taken, when there is about 10 per cent. in bark and 8 per cent. in wood, this being the second peak. The figure for the previous year's shoot in April is slightly higher than this, indicating that the starch content does not fall until the bud swelling period is well advanced.

This two maxima curve has been found by several observers (Fischer (10), Cameron (3), Price (32), for example). There is thus a period of hydrolysis in the one year old twig prior to the formation of the new shoot (which contains a minimum percentage of starch) followed by a gradual accumulation of starch throughout the growing season, the rate of which increases at the onset of leaf fall. The maximum is reached at the end of October before all the leaves have fallen and hydrolysis then proceeds until mid-winter.

It will be useful here to examine the sugar curves (Figs. 3, 4, 5) in relation to starch content. The peak value for reducing sugars in June corresponds to the starch minimum. Presumably some of the starch present in the previous month in the one year shoot has been transferred as reducing sugars to the new shoots. This sugar decreases in the bark during the growing season due chiefly to use in growth and respiration, while starch increases, the leaves being the main source of supply for this accumulation. In the wood no significant change in starch occurs, though the sugars decrease as in the bark. Towards the end of the full growth period (September), reducing sugar, sucrose and starch all increase in bark and wood. The starch maximum is reached by the end of October but the sugars continue to increase, at the expense of the starch already in the stem, as the trees are by now defoliated. This hydrolysis continues until in January a sugar maximum and starch minimum occur simultaneously. Resynthesis of starch until April corresponds to a decrease in the sugars; the subsequent starch hydrolysis till June, though accompanied by a rise in reducing sugar is not accounted for by the latter, as the sucrose also decreases by an equivalent amount. The sugar (reducing) and starch relations are however reciprocal from the end of October, through leaf fall and the dormant period, until about the beginning of March.

The hydrolysis of starch during the cold period, a change which Hooker (17) has also found in spurs, may be useful for the prevention of freezing, owing to the consequent higher sap concentration, but whatever may be the advantage of the change, there is no explanation of its cause, though Fischer (10) suggests the change in temperature itself as the chief factor here, while Mitra (25) observed changes in the pH to occur at this time, by which he accounted for variation in enzyme activity.

Hemicellulose (Fig. 7). The hemicellulose content in the wood is approximately twice that of the bark, a difference also noted by Proebsting (33). The value for wood in June is about 18 per cent. dry weight and increases to 24 per cent. at the end of August. In the bark the June value is 10 per cent. and there is no change through July and August. This difference between bark and wood content is the reverse of that which occurred for starch at the same period; there the wood content remained at 4 per cent. for two months while the bark content rose from 8-10·5 per cent. It thus appears that during the elongation period the surplus sugars from photosynthesis which pass to the stem are transformed into starch in the bark and hemicellulose in the wood (for there is no increase in starch here). As the bark is the chief organ of translocation of carbohydrates, it might be expected to accumulate the more labile form of higher carbohydrate (i.e starch) rather than hemicellulose at this time. This period is also one of hardening for the wood, so that if hemicelluloses are structural in function they would tend to increase here. Throughout the year the percentage of starch and sugars (i.e. more labile substances) is higher in bark than wood, while the reverse occurs for hemicellulose content, a fact which supports the view expressed above.

Apart from increase in the hemicellulose of the wood, during the growing season the variations are not great. The content falls from the October maximum of 24 per cent. to 21·5 per cent. during that month, after which there is no significant change. In the bark the value is approximately constant for most of the year, with one rise from 10-12·5 per cent. dry weight in September followed by a fall to 10 per cent. again in October. The slight rise for bark and wood to a peak value in January cannot be considered sufficient evidence for the presence of a second maximum in the seasonal cycle, although the smoothness of the curve is a point in favour of the validity of the peak, as also the fact that grass and arable plot figures show the same tendency. The similarity between the hemicellulose and lignin curves, in addition to the fact that the seasonal fluctuations are relatively small, points to the conclusion that in terminal shoots this fraction is mainly structural in function.

Total Carbohydrates (Fig. 11). Under this heading sugars, starch and hemicellulose are included, while a graph for starch and sugar only is also given (Fig. 10) and termed "Reserve carbohydrates". Cellulose has been excluded because it is obviously a structural substance.

The curves in both groups are similar in form except during the summer and early autumn (June to end of September) when the reserve carbohydrate curves reflect the starch differences; the latter are masked when hemicellulose is included, as under total carbohydrates.

The total carbohydrate content (including hemicellulose) is at a minimum in June, increasing steadily in both tissues until the end of October, bark varying from 28·4-20 per cent. and wood 35·4-22·2 per cent. Grass and arable plot figures are similar. The high value is retained in the bark until the last determination in February; the April value of the previous year is lower, but the minimum value occurs in June; in the wood the decrease from the high winter value begins in January. The winter minimum observed for starch is masked by the higher sugar and hemicellulose contents found at this period.

The total amount of carbohydrate thus appears to increase from the early stage of the young shoot until after leaf fall. During the winter, interchange among the various fractions occurs but the total remains high. In April and May the total content decreases in the stems (which are now nearly one year old) in spite of the observed increase in reducing sugar. This decrease may be due to the transference of some of the starch as sugar into the swelling terminal buds which were not included in the analyses here, or to destruction of the sugar by increased respiration.

Total reserve carbohydrates (sugar and starch) in bark vary from 17·4-11 per cent. dry weight, in wood 14·2-5 per cent., that is, a higher content in the bark, but if hemicellulose is included, higher total content is found in the wood.

Cellulose (Fig. 8). There is a low value for bark and wood in June, followed by a rise in July after which the fluctuations are insignificant in the wood, but are greater and more variable in the bark. The percentage content of both tissues is roughly the same—between 2 and 3 per cent. Grass and arable plot figures are similar. (Note: The divergence of the first grass and arable plot values for wood cannot be explained; they were the first samples analysed, but each had a duplicate check.)

Lignin residue (Fig. 9). Lignin content is about 15 per cent. dry weight higher in wood than bark, as would naturally be expected. In wood, variation from 43-27 per cent. is found, in bark 27-17 per cent. The content rises during the growth season when wood vessels are being laid down and enlarged, and in the bark, "sheath" cells increasing; the figures for lignin in the wood thus correspond to those obtained for percentage dry wood in shoots by Karmarkar.

LEAVES.

Alcohol soluble matter (Fig. 12). The percentage of alcohol soluble matter here is higher than in the other tissues, as would be expected, and remains at about 45 per cent. dry weight throughout the period of the leaf's activity (June to end of November). The fact that this fraction is mainly composed of non-reserve materials would account for this. A second point which may be noted here with regard to the last sample which might have been expected to show a decrease is that the majority of leaves collected here had to be taken from the upper part of the twigs and the balance between old and younger leaves (maintained by selection over the whole shoot, in previous samples), was thus disturbed. This point should be remembered throughout the discussion of the other fractions in the last sample.

Reducing Sugars (Fig. 13). In these determinations the errors due to diurnal variation, which is probably greater than that in the stem, has been avoided as far as possible by sampling in fair weather and always at the same time (10.30-11.30 a.m.). The regularity of the results suggests that they have some significance.

The first sample of leaves taken in June (full grown) showed a maximum value for reducing sugars. Throughout the growing season and until the end of September the sugars decrease, the range being from 5·2 per cent. to 2·64 per cent. dry weight. They thus reach a minimum at the same time as the reducing sugars in bark and wood. This minimum is followed by an increase until the end of November when the majority of leaves had fallen, and the last sample was taken. Grass and arable plot figures agree.

Sucrose (Fig. 14). The sucrose value in June is approximately 1·4 per cent. dry weight, rising during July to 1·8 per cent., then falling to a minimum (1·2 per cent.) at the end of August. The concentration rises again as for reducing sugars, but the maximum is reached a month earlier for the grass plot and two months for the arable plot. This would appear to be due to a mobilization, preliminary to removal of sugars to the stem, during November (leaf fall), when there is a sharp decline to a second and lower minimum, while reducing sugars are still increasing. The sucrose decline may be due to a lower rate of sucrose synthesis, or to inversion to reducing sugar in the leaf, or again to transference of sucrose to the bark, where both sugars are accumulating at this time.

Durant (9) has made some observations on the carbohydrate content of leaves, dividing the foliation period into two parts, the first, a period of carbohydrate synthesis and polymerization, when all forms of carbohydrates increase, continuing until the end of the summer, and the second, one of simplification and hydrolysis during which sugars continue to increase but starch and higher carbohydrates decrease. According to his results, therefore, the sugars increase steadily through both periods, which has not been found to occur here, a distinct minimum appearing instead for both sugars (reducing sugar in September and sucrose in August).

The sucrose curves for grass and arable plots do not agree so closely here as for other constituents, the chief divergence occurring in the position and height of the autumn maximum, which appears at the end of September for the arable plot and of October for grass plot trees. The difference of a month between the collection of consecutive samples of course makes it possible that the highest peak for the grass plot was not determined and may have occurred in mid-October.

Total Sugars (Fig. 15). These show a two maxima curve, with a high peak in June, about 6·5 per cent., and a second at the end of October 6 per cent., that is, just prior to leaf fall; the first is presumably due to the greater activity of the young leaf, and the second either to the hydrolysis of starch preliminary to the autumn migration before defoliation, or simply that sucrose is being formed in preference to starch as an alternative form of storage carbohydrate, more suitable at this period.

Starch (Fig. 16). The figures show a steadily decreasing starch content from the first sampling in June until the end of October, after which a slight increase occurs for the last sample in early December; the younger age of the leaves in this material, previously mentioned, may account for this. The percentage falls from about 8 to 4, showing that in the falling leaves there is still some starch left unhydrolysed. Thus during the growth period reducing sugars and starch decrease, but in September and during the beginning of defoliation (late October) the reducing sugar rises while starch content continues to decrease.

If the equilibrium between leaves and stem tissues (bark and wood) is considered (Fig. 19), we find a decrease in reducing sugars in bark, wood and leaves during the growing period (sucrose is at a minimum in bark and wood, but rises and then falls in the leaf). Increase of starch, however, occurs in the bark at this time with a decrease in the leaves. The starch appears thus to be transferred to the stem instead of being stored in the leaf as at first. In autumn starch and sugar contents rise in the stem, and sugar in the leaves, but the starch continues to decrease here.

There appear to be few data for seasonal analysis of starch in leaves from apple shoots over long periods by other investigators, though Murneek has examined the changes in spur leaves during summer. Harvey's (13) results for total carbohydrates and pentosans in summer have been mentioned previously. Murneek and Logan (26a) analysed leaves from one, two and three year wood in October and November, estimating starch, sugars and hemicellulose. Their results for two consecutive years differed considerably and they point out that "modifications induced by weather are often of greater amplitude than a possible seasonal trend". Further work with analyses at shorter intervals than one month, with concurrent microchemical tests should be of interest.

Hemicellulose (Fig. 17). The curves for hemicellulose are less regular in the arable plot trees than in the grass plot, but both sets show an increase in content throughout the period of the leaf's activity. During the first three months, June to September, we thus have decreasing sugar and starch contents and increasing hemicellulose. In October the sugars increase, the other curves continuing as before. It may be noted that the rate of starch decrease is about twice that of the hemicellulose rise.

The form of the curves again suggests that the function of this fraction is mainly structural.

Total Carbohydrates (Fig. 18). These curves show a similar trend whether or not hemicellulose is included, that is, a gradual fall from June to the end of September (total 22·4-17·5 per cent., reserve 14·4-10·1 per cent.), and an approximately constant minimum value for the last two months. Thus during the later months the content of the leaves was at its lowest and that of the stem approaching its maximum, due, of course, to translocation from the leaf. It is difficult to attempt an explanation of the interchange of the various substances between leaves and stem, unless information about the rate of photosynthesis itself is obtainable. For instance the decreasing starch content found in the leaves as the season advanced, may be explained by a decreasing photosynthetic rate or by increased rates of inversion, or translocation. Very little work has been done on photosynthesis of apple leaves *in situ*, and, of course, elimination of sources of error is particularly difficult here, but Heinicke (16), 1933, has carried out a few experiments on ringing effects in this connection, and the application of his method to other problems should be of interest.

SUMMARY OF THE COMPOSITION OF THE TERMINAL SHOOTS AT CERTAIN IMPORTANT STAGES OF DEVELOPMENT DURING THE YEAR.

The condition of the shoots during the five periods corresponding to various important physiological stages is shown diagrammatically by the graphs in Fig. 19, where the cycles of all the carbohydrate fractions have been plotted to the same scale. To simplify the diagrams, data for arable plot only are given.

1. *Period of shoot elongation* (June, July and part of August).

Bark and Wood. The first sample of the young shoots (about seven inches long) was taken in June, so the cycle is best considered with this period as a starting point. At the beginning of the period the young shoots are at a maximum water content, maximum alcohol and water soluble material, and the reducing sugars have also reached their first (but lower) maximum. The sucrose, starch, hemicellulose, cellulose and lignin content are at minimum points in their cycles.

Throughout this period the alcohol soluble and water soluble matter and reducing sugars decrease, the sucrose remaining at a minimum value. Starch increases in the bark only and hemicellulose in the wood, while lignin and cellulose increase in both tissues. The total carbohydrate (sugar, starch and hemicellulose) content rises for both tissues, due of course to the activity of the leaf.

Leaves. The reducing sugars and starch decrease steadily; sucrose rises through July and then falls to a minimum at the end of August; hemicellulose, however, increases, though not as rapidly as the other constituents decrease.

Total carbohydrates therefore show a decrease through this period. Alcohol soluble content shows no change through the whole season, and will not therefore be mentioned again when referring to leaves.

2. *Period of Full Growth* (end of August, September and most of October).

Bark and Wood. The alcohol soluble matter and reducing sugars continue to decrease, the latter reaching their minimum values by the end of September, and the former in October; the sucrose content remains low; this point also coincides with the reducing sugars minimum in the leaf. No significant change occurs after this in the alcohol soluble matter. Both sugars then (at the end of September) begin to accumulate, while starch increases in bark and wood, so that these carbohydrates are all contributing to the rise in the total carbohydrates. Hemicellulose, however, reaches its maximum in both tissues by the end of September, and falls slightly during October, after which the changes are not significant. The stem is thus collecting food material from the leaf at a fairly rapid rate towards the end of this period.

Leaves. Here the reducing sugar reaches a minimum at the end of September as in the stem, and sucrose a month earlier. Both sugars then rise (except sucrose in the arable plot, while starch continues to decrease through the whole period and hemicellulose increases (though with fluctuations for the grass plot).

3. *Leaf Fall* (end of October and November).

Bark and Wood. Reducing sugars and sucrose continue to rise throughout, with higher gradient in bark than wood. The starch content also rises but reaches its maximum in both tissues at the end of October, and then decreases. Some of the starch is thus hydrolysed before the close of leaf fall; possibly because the rate of transfer of sugar from the leaf has diminished and the sugar→starch reaction in the stem reverses. Total carbohydrates are approximately constant.

Leaves. Reducing sugars increase to a maximum by the end of the period, which includes the last sample, taken when the majority of leaves had fallen. The sucrose decreases through November. The starch decreases to a minimum (still 4 per cent. dry weight) at the end of October; the slight rise in the last sample for November has been previously discussed. The total carbohydrate content remains at a minimum.

4. *Dormancy* (December, January, February, March).

Bark and Wood. The changes occurring here are, of course, due to interaction between constituents already in the stem, as the leaves have fallen. The treatment of the terminal shoots here, almost as if they were independent units, when discussing the relation between the sugar and starch may be criticized as omitting to take into account the vertical translocation of constituents to and from the two year old portion below. However, both from Mitra's work (25) and Traub's (44) results on shoots of apple trees, there appears to be a great similarity between one and two year shoots, both in composition and behaviour of the chemical constituents. This method of discussion in the present case would therefore appear to be justifiable.

The dormant period is characterized by a rise in reducing sugars and sucrose (in both tissues) to a maximum, reached at the end of January. At this date, the starch, which has continued to decrease from November, reaches its minimum, so that changes of sugars and starch appear reciprocal.

During the second half of the period the reverse reaction occurs, starch being synthesized at the expense of the sugar. No sample was analysed for March, the last sample being taken on February 28th; the value for April (in the previous year) is slightly higher than that for February, but whether or not the starch synthesis continues through May cannot be stated.

The total carbohydrates show no significant change, maintaining their high value throughout the period.

5. *Bud Swelling* (April and May).

There is only one sample to represent this period, taken on April 18th, 1932. At this point, the alcohol soluble matter and reducing sugars are at a low figure in bark and wood, as compared with the content of the first sample of young shoots in June (the next estimation after April). Sucrose values are higher (particularly in bark) than those for June; total sugars slightly higher in bark and lower in wood. Starch and total carbohydrates are at a considerably higher content in both tissues than in June, while hemicellulose, cellulose and lignin also are present in greater amounts. A discussion of the condition during the whole period is obviously impossible from the results of this one sample.

COMPARATIVE SUMMARY FOR BARK AND WOOD.

The cycles for bark and wood are very similar in form; the chief differences have already been discussed—the difference in amount of decrease of sucrose in spring, and the longer period of minimum starch content for wood than bark in summer, with correspondingly shorter period of hemicellulose minimum. The dates on which maximum and minimum contents occur are otherwise similar for bark and wood.

The following Table of the contents at the maximum and minimum points serves to illustrate the relative proportions of the constituents in the two tissues. The figures have been given for the grass plot only; those for arable can be seen in the summarizing graph (Fig. 19). Where two maxima occur in the cycle the figures are given in order of sampling and representation in the graphs.

Table of Maximum and Minimum Contents. Percentage Dry Weight.
Grass Plot.

		Alcohol Soluble.	Reducing Sugar.	Sucrose.	Total Sugar.	Starch.	Hemi-cellulose.	Total carbohydrate. (Total sugars + starch and hemicellulose.)	Cellu-lose.	Lignin.
Bark	Maxima.	39·5	2·57 4·30	2·77	6·98	9·71 12·92	12·62	27·40	3·89	27·4
Wood ..		29·1	1·45 2·15	1·15	1·84 3·37	9·48 12·73	24·06	34·50	3·24	43·7
Leaves ..		48·1	4·82	1·80 2·27	6·72	9·15	9·37	22·40	—	—
Bark	Minima.	24·5	1·74 1·82	0·49	2·21	7·73 7·55	9·51	21·00	1·20	17·9
Wood		7·5	0·59 0·51	0·08	1·02 0·59	3·75 7·98	18·09	22·20	1·66	27·4
Leaves ..		45·2	2·64	10·01 0·87	4·10	4·10	6·98	18·58	—	—

From the above Table it can be seen that the content of the bark is higher than that of the wood in alcohol soluble matter, reducing sugars and sucrose. The curves for the seasonal cycles show that this relation persists throughout the year and confirms the accepted views on the translocation functions of the bark.

In the starch cycle the values for spring and autumn maxima are similar for bark and wood, and this applies to the winter minimum. In June, however (summer minimum), and through the growing season the bark has a considerably higher content, 7·7 per cent. for bark and 3·8 per cent. for wood. This higher content might be expected in the bark of young shoots, and also the accumulation of starch through the growth period (when sugar is being steadily transferred from the leaf), if one considers that the bark is the chief organ of distribution of carbohydrates. The very rapid accumulation of starch in September and October by the wood (4-12·5 per cent.) in contrast to the rise of from 10·5-12·5 per cent. in the bark may simply be due to the fact that the bark content was already high, while the wood parenchyma appears to be acting as a reservoir for the increasing flow from the leaf, just prior to leaf fall.

The hemicellulose content of the wood is nearly twice that of the bark. This fact, and also that it increases only in the wood during the elongation period is in accordance with the view (Murneek (26)) that it is closely connected with the formation and enlargement of the xylem tissues themselves. The figures obtained are of the same order as those found by Traub (44) and Proebsting (33) where they are comparable.

Cellulose contents are much the same for bark and wood, and quite low (maximum 4 per cent.) in both tissues.

The lignin is naturally much higher in the wood (maximum 44 per cent.) than bark, which, however, contains a considerable amount (27-18 per cent. range): the bark content is largely due to the sheath cells which occur in the cortex.

COMPARISON OF RESULTS FROM GRASS AND ARABLE PLOTS.

The cycles of the various carbohydrate fractions and of lignin for the trees from the two plots are very similar, the only differences of note being the slightly higher contents of reducing sugar and starch in the bark of the arable plot trees from April to the end of November, these being less marked from September onwards—in the wood there is a slightly lower starch content for arable than grass plot from April until the end of August.

Since the differential cultural treatments only resulted in a slightly lower nitrogen content of the trees from the arable plot, one would not expect to find a significantly higher carbohydrate content in these trees; such a condition would only have occurred if the nitrogen content had fallen sufficiently low to become a limiting factor, as in Group IV of Kraus and Kraybill's series.

THE CARBOHYDRATE AND NITROGEN RELATION IN THE SEASONAL CYCLES OF TERMINAL SHOOTS.

Ratios of carbohydrate/nitrogen have been plotted in Fig. 20 for total sugars, starch and reserve carbohydrates (starch + sugar) compared with total nitrogen, and in Fig. 21 the same carbohydrate fractions are shown relative to total non-protein nitrogen. In this second set, non-protein nitrogen was selected because this fraction represents the more simple components of protein synthesis, or degradation, and might thus be expected to exhibit fluctuations comparable with or similar to those of the more simple carbohydrates. The curves are given for bark, wood and leaves and are briefly discussed below. The most notable result appears to be that the grass plot ratios are generally higher than those of the arable plot, which is the reverse of the result obtained for the carbohydrate fractions considered without reference to nitrogen.

Carbohydrate/Total Nitrogen (Fig. 20).

Bark. The curves for starch and "starch + sugar" are similar and the characters of these are dominated by the variations in the starch content. The importance of this latter fact should be borne in mind when carbohydrate/nitrogen ratios are considered. There is a low June figure which rises gradually until the end of September, falling again almost to the first minimum by the end of January. Thus in June, the young shoots have relatively high nitrogen and low carbohydrate (the reducing sugar peak is masked here by the low starch content); the period July-August, during which fruit buds are laid down, corresponds to the point where the ratios begin to show high values. By the beginning of leaf fall (October-November) carbohydrates have accumulated to a still higher level, and nitrogen, though increasing is, in comparison, moderately low. "Starch + sugar" content decreases slightly after leaf fall while nitrogen continues to increase, and the value of the ratio therefore also decreases. A minimum is finally reached in January, which corresponds to a high nitrogen and a moderately high carbohydrate content.

Total sugars/total nitrogen ratio shows a very gradual increase from June until the last estimation in February. Thus during November, December and January, the sugar ratio increases while the starch ratio decreases.

Wood. Starch and "starch + sugar" curves are again very similar, and their general form is that of the seasonal cycle for starch alone (Fig. 6), once more illustrating the dominance of starch in considering carbohydrate/nitrogen ratios in shoots. There is thus a minimum June figure, with low values for July and August, and a considerable rise to a maximum at the end of October after which the curves fall more gradually. The low values for July-August period are of interest in connection with fruit bud formation and it would appear that if a high carbohydrate condition is required for this then the necessary reserves are present in the bark tissues. The grass plot figures are higher than the arable except for the June sample.

Total sugars/total nitrogen shows a gradual rise from June to January (as in bark); the grass values are very slightly higher in autumn.

Leaves. Ratios for starch and "starch + sugar" decrease slightly until about the end of September when there is a very slight rise due to a decrease in the nitrogen values. Total sugar ratios remain approximately constant.

Carbohydrate/Total Non-Protein Nitrogen (Fig. 21).

Bark and Wood. The cycles for the three sets of ratios ("starch + sugar"), starch, total sugars/non-protein nitrogen need not be discussed in detail as they resemble the curves for the corresponding sets related to total nitrogen. The similarity is more marked for the wood series, due to the fact that the separate cycles for total nitrogen and non-protein nitrogen agree more closely in the wood than bark. The higher ratios for the grass plot appear in all the graphs; in the total nitrogen ratios this feature was not shown clearly, but the fact is quite obvious in those for non-protein nitrogen.

Leaves. "Starch + sugar" and starch ratios show a decline from June till November by about 50 per cent. of the original values. This drop is almost entirely due to decreasing starch content, as the non-protein nitrogen ratios vary only slightly. The peak value for the grass plot in September can be explained from the sugar curves, and is due to the difference in the dates of the maxima for the two plots; there is also a fall in the non-protein nitrogen of the arable plot at this point with a rise in the content of the grass plot, which would increase the difference between the two ratios. During the first three months (June-August) the ratios are higher for the grass plot.

Total sugar ratios, which resemble the curve for total sugar alone, show a similar September peak for arable plot trees.

The effect of the sugar changes is seen in the "starch + sugar" ratios, and is not masked by the starch cycle, as occurred in the bark and wood series. This is due to the relatively higher concentration of total sugars in the leaves.

The main conclusions to be drawn from the consideration of these two sets of ratios appear to be as follows: (1) The value of each ratio shows seasonal variations and is rarely constant over two consecutive months; (2) the ratios for the carbohydrates (particularly starch) to non-protein nitrogen are consistently higher for grass than arable plot trees in both bark and wood, except for the first sample of young shoots taken in June. This difference also occurs in the wood for starch and "starch + sugar"/total nitrogen, and in the leaves for starch ratios with both nitrogen groups, through June, July and August; (3) the carbohydrate/nitrogen ratios in bark and wood are dominated by the changes in starch content, the resulting cycles having a similar form to those for this fraction alone. This does not occur in the ratios for the leaves, owing to the higher sugar content in the leaves.

CONCLUSIONS.

1. Seasonal variations occur in all the constituents examined (alcohol soluble matter, sugars, starch, hemicellulose, cellulose and lignin). These variations are found in both bark and wood and are greatest at the bud swelling and defoliation periods.

2. The cycles for the constituents in bark and wood are generally similar, though differences in absolute amounts occur.

3. The application of the spring nitrate dressing to the grass plot did not appear to affect the form of the seasonal cycles, as the curves for the two plots show very close agreement. The concentration of the constituents in the trees is also approximately the same for both plots, except reducing sugars which are present in larger amounts in the arable plot trees during spring and summer: at the same period the starch content was slightly higher in the bark of arable plot trees than grass plot trees, but lower in the wood of the arable plot trees. The carbohydrate/nitrogen ratios, however, are higher in the trees from the grass than those from the arable plot.

4. The main features of the cycles for each constituent may be summarized as follows:

(i.) *The alcohol soluble matter* of young shoots in June is at a maximum and decreases steadily throughout the growth period to a minimum in October, after which no change occurs; bark contents range from 40-25 per cent. dry weight, wood 29-8 per cent. The curves are similar to those obtained by Karmarkar for water soluble matter. Leaf content remains at about 45 per cent. through the season. Grass and arable plot samples gave similar results.

(ii.) *Reducing sugars* are highest at the end of January and at a minimum in April (no May sample). A second smaller increase occurs in the June sample, greater for bark than wood, and a slight decrease through the growth period until September. Bark content ranges from 4·3-1·7 and wood 2·2-0·5 per cent. The leaf content falls from about 5 to 2·7 per cent. by the end of September and then rises. The arable plot samples have a higher content than the grass in spring and summer.

(iii.) *Sucrose* content is at a minimum in June and only begins to rise in the autumn, in the bark from 0·5-2·8 per cent. and in wood 0·08-1·2 per cent. The maximum is reached at the same time as that for reducing sugars. In the leaves a minimum is found in September, a maximum in November and then a sharp decline at the end of leaf fall. Grass and arable plot figures are similar.

(iv.) A minimum *starch content* is found in the young shoots in June: 7·5 per cent. dry weight bark and 4 per cent. wood. Starch increases only in bark during the elongation period. In September both bark and wood tissues accumulate this substance, a maximum in each case being reached by the end of October (12·9 per cent. bark and 12·7 per cent. wood). A second minimum (7·5 per cent. approximately for both) occurs in January; this is followed by a rise, which in the April sample reaches 9·7 in bark and 9·5 per cent. in wood prior to the spring hydrolysis. This starch rise is associated with a corresponding fall in sugar values. No significant difference is shown between grass and arable plots. The starch content of leaves decreases from 8-9 per cent. in June to about 4 per cent. dry weight at the onset of leaf fall.

(v.) The *hemicellulose content* rises in wood from 18-24 per cent. between June and August, but in the bark only a slight rise occurs in October: the wood content is about twice that of the bark: grass and arable plot figures are similar. In leaves the value rises throughout the season from about 7-9 per cent. dry weight.

(vi.) *Total carbohydrates,* including hemicellulose, have a single minimum in June and gradually rise till the end of October, after which the high value is maintained. Bark ranges from 27·4-21 per cent. dry weight, wood 34·5-22 per cent. Grass and arable figures are similar. In the leaves a decrease occurs from June to September and this low value is maintained afterwards.

(vii.) *Cellulose* values fluctuate in bark more than in wood. There is a low value in June and a rise in July, after which there is an approximately constant content. Bark and wood have similar values, ranging from 4-1 per cent. Grass and arable plot figures are not significantly different. No leaf analyses were made.

(viii.) The *crude lignin* fraction is higher in wood than in bark, varying from 44-27 per cent. in wood and 27-17 per cent. in bark. A rise occurs in the young shoots during the period of active growth. Grass and arable plot figures are similar. No leaf analyses were made.

5. Certain observations may be made on these results in connection with the respective functions of the constituents. The content of the bark with regard to sugars is consistently higher than that of the wood. Starch content is also higher in the bark of the young shoot throughout the growth period. These facts agree with the accepted view of the bark as the chief organ of carbohydrate translocation.

Sucrose content is low and always below that of the reducing sugars. The seasonal changes for sucrose however are relatively as great as for reducing sugars. This, with the facts that minimum sucrose occurs in June during the active growing period, when reducing sugars have increased and that sucrose increases in autumn when reducing sugars and starch are accumulating from the leaf, suggests that sucrose in the shoots acts as a primary reserve, rather than simply a medium of translocation.

The occurrence of a two maxima curve for starch is observed and confirms previous results. There is no complete disappearance of starch in winter at any time, as has been stated from some micro-chemical investigations. The term starch is here applied to the product obtained by enzyme digestion followed by acid hydrolysis and the quantity might not necessarily correspond to that observed microscopically.

Decrease of reducing sugars in summer occurs simultaneously with an increasing starch content while the reverse occurs after leaf fall (before which both constituents and also sucrose accumulate). The starch minima and corresponding reducing sugars maxima occur at the same dates. The hydrolysis and resynthesis of starch during the dormant period and early spring, is associated with corresponding reducing sugar variations in a reverse direction. The sucrose curves during this period follow the course of the reducing sugars, so that the total carbohydrate content remains high. In the leaf starch content falls steadily through the season.

The hemicellulose results show that although there is a seasonal variation, this is not great and there is always a considerable proportion present; this and the similarity of hemicellulose to the lignin curves in bark and wood support the view that this substance, as obtained by the analytical methods described, has mainly a structural function rather than one of a primary reserve. Further division of the fraction appears to be necessary.

The increase in cellulose and lignin content during the growing season, and the lack of further significant variations, was to be expected from the known structural functions of both these constituents.

6. The ratios of the various carbohydrates to nitrogen and non-protein nitrogen show that there is very rarely a steady value for the ratio over two consecutive months. The curves of the ratios are similar for grass and arable plot trees, in form, but the values are higher for the grass plot, for the starch, and sugar / non-protein nitrogen ratios in bark and wood; for the starch / total nitrogen in wood; and for starch / both nitrogen groups during the summer months in the leaves. The seasonal cycles of the ratios in bark and wood are dominated by the changes in the starch fraction. In the leaves the higher sugar values modify this effect.

SUMMARY.

An investigation of the carbohydrate and lignin contents in terminal shoots of apple trees, variety Newton Wonder, is described: the fractions examined were alcohol soluble matter, sugars, starch, hemicellulose, cellulose and lignin.

Two sets of trees (eight per set), one under grass and the other under clean cultivation, were employed, those on the grass plot receiving a spring dressing of sodium nitrate (5 cwt. per acre).

Monthly collections were made of samples from each of the two plots; each sample comprised 50-60 shoots (6-7 per tree). Sampling was carried out usually on consecutive days, for the two plots, and between 10.30 and 11.30 a.m. Separation was made into bark and wood, and when leaves were present, into wood, bark + petioles, and laminae.

Seasonal cycles were found to occur for all the constituents examined, and their significance is discussed. The differences between contents of wood and bark and between the results from the two plots are also considered.

Observations are made on the relation between the carbohydrate and nitrogen (total and total non-protein nitrogen) cycles, and the various ratios for these constituents are graphically represented.

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APPENDIX.

CHEMICAL METHODS.

Alcohol soluble matter and sugars. The samples, consisting of 60 gm. fresh weight, had been preserved in sufficient alcohol to produce an 85 per cent. solution, allowance being made for the percentage moisture in the material (about 50 per cent. bark and wood and 70 per cent. leaves). This alcohol was poured off and the sample extracted with fresh 85-90 per cent. alcohol in soxhlet extractors for about eighteen hours. After cooling and bringing the volume of the joint extracts to a litre, the alcohol soluble portion was determined from 50 c.c. portions, which were evaporated on a water bath and then dried to 70° C. in an oven overnight.

The remaining extract was distilled under reduced pressure at 50-60° C. and the residue (20-30 c.c.) washed into a beaker with hot water, cleared with about 20 c.c. (saturated solution) neutral lead acetate and deleaded with potassium oxalate, and made up to 500 c.c. The reducing power was determined with Shaffer and Hartmann's (37) "combined reagent" the copper oxide being filtered off, dissolved in acid ferric sulphate solution and titrated against N/20 potassium permanganate solution. This figure after conversion of the copper value to glucose gave the concentration of reducing sugars in the extract.

A 50 c.c. aliquot was then hydrolysed after addition of 5 gm. of citric acid, by boiling for ten minutes. After neutralization with caustic soda solution, and dilution to 100 c.c., the reducing power was again determined. This represented the value for total sugars (sucrose and reducing sugars) in terms of glucose. By subtracting the previous reducing sugar value and mutiplying the resulting figure by 0·95, the concentration of sucrose was obtained.

Starch. The residue from the alcoholic extraction was dried at 70° for twenty-four hours, weighed and extracted in soxhlets with ether for further removal of any fats. After drying again it was ground in a mill, and finally by hand in an iron mortar till the material passed through a 100 mesh sieve.

Four gm. of material were used for each starch estimation. 100 c.c. of hot water were added to the sample in a conical flask, the mixture brought to the boil and then autoclaved for two hours at 120° C. It was then cooled, and 10 c.c. of an approximately 1·5 per cent. solution of malt diastase (B.D.H.) were added. A blank was also run with enzyme solution alone. The flasks were placed in a water bath and brought to a temperature of 70° C. in twenty minutes, held there for half an hour, and then at 80° C. for ten minutes, after which the water in the bath was boiled for two or three minutes, when the temperature in the flasks rose to about 95° C. This destroyed the enzyme and produced further gelatinization.

After cooling to about 40° C., a second addition of 10 c.c. of approximately 0·7 per cent. diastase solution was added to the mixture in the flasks and to the blank. They were returned to the water bath and maintained at a temperature of 55° C. for an hour and then brought to the boil.

The mixture was filtered, washed with hot water and the volume of filtrate made up to about 200 c.c. 10 c.c. of concentrated hydrochloric acid were then added, and the solutions hydrolysed for two and a half hours in a boiling water bath with reflux condensers. After neutralizing with sodium hydroxide they were cleared, deleaded, filtered and made up to 500 c.c. The reducing power was estimated by Shaffer and Hartman's combined method, using N/10 thiosulphate to determine the iodine value for the copper reduction. The glucose figure × 0·9 was termed "starch".

"Hemicellulose." 4 gm. of the alcohol extraction residue were taken for each estimation. 150 c.c. of 2 per cent. hydrochloric acid were added to the material in 750 c.c. conical flasks (large size, because the mixture froths). The mixture was boiled, using reflux condensers, for five hours. It was then filtered on to a weighed paper, washed with about 150 c.c. hot water, and the filtrate neutralized with concentrated sodium hydroxide; cleared and deleaded, and the reducing power determined as described for starch. The resulting glucose value × 0·9, minus the starch figure, was taken as a measure of "hemicellulose".

Cellulose. 1 gm. of the dried and weighed residue from the hemicellulose extraction, which had been ground again and sieved, was treated with 10 c.c. 80 per cent. sulphuric acid in the cold for two and a half hours; 150 c.c. distilled water was then added and the mixture autoclaved for one hour at 120° C. After filtering on to weighed paper, and neutralizing the filtrate (clearing was unnecessary here), the reducing sugar was estimated as described for starch. The factor 0·9 was again used to convert the glucose value to cellulose.

Lignin residue. The residue from the cellulose extraction was dried and weighed. One of each pair of duplicate samples was ignited to determine any inorganic impurity which should be subtracted from the weight of the residue. This impurity was found to be negligible and ignition was discontinued later.

Allowance for moisture. Moisture determinations were made on the alcohol insoluble residue after grinding and the percentage deducted from the air dry weights taken for the starch and hemicellulose determinations. All the calculations, however, are based on the original dry weight of the plant material before extraction with alcohol. Therefore, after subtracting the percentage of moisture from the air dry weight taken, addition of the percentage of alcohol soluble matter had to be made. The final result gave the equivalent to 4 gm. air dry alcohol extracted material, in terms of unextracted, dried plant tissue.

Accuracy. Agreement between duplicates varied slightly with the substances estimated. For twelve sets average differences between duplicate samples in terms of the percentage of the mean value for each pair are as follows:

		% Error.		% Error.
Alcohol soluble	Bark	2·49 ± 0·525	Wood	1·63 ± 0·406
Reducing Sugars	,,	2·17 ± 0·373	,,	1·65 ± 0·129
Total Sugars as Glucose ..	,,	2·23 ± 0·521	,,	1·72 ± 0·448
Sucrose (by difference × 0·95)	,,	2·85 ± 0·734	,,	4·80 ± 0·700
Starch	,,	2·49 ± 0·443	,,	2·75 ± 0·456
Hemicellulose	,,	2·75 ± 0·470	,,	2·21 ± 0·495
Cellulose	,,	3·21 ± 0·666	,,	3·61 ± 0·807
Lignin	,,	3·94 ± 0·841	,,	3·03 ± 0·536

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ON THE BIOLOGY OF SOME TORTRICIDAE (LEPIDOPTERA) INFESTING FRUIT TREES IN BRITAIN

R J.M.C.

1. *CACOECIA* (*TORTRIX*) *PODANA* SCOP.*

By G. L. HEY† and F. J. D. THOMAS‡

† Advisory Department, Murphy Chemical Company.
‡ East Malling Research Station.

I. INTRODUCTION.

ONE of the earliest records of *Cacoecia (Tortrix) podana* Scop. in Britain is that of Wood (42), in 1854. He figures the moth under the synonym of *Lozotaenia fulvana* (Oporana, St.), the Great Hook-tipped and records it from hedges and gardens near London. The Entomological Societies of Oxford and Cambridge in 1858 record the moth in their Accentuated List of British Lepidoptera (7) as *Tortrix pyrastrana* Hub. Stainton (35), in 1859, describes it as *Lozotaenia fulvana,* giving *Tortrix pyrastrana* Hub. as a synonym and in the same year Wilkinson (41) gives an account of the species under the synonym of *Lozotaenia fulvana* Schiff. (?) In 1868 Morris (28) describes the moth as *Tortrix pyrastrana*. Since then Meyrick (23), in 1895, has described the species as *Cacoecia podana* Scop. while Barrett (3), in 1905, has employed the synonym *Tortrix podana* Scop. Hein. Other brief records are those of Theobald (37, 38), Deakin (6), Adkin (1), Huggins (18), Fassnidge (8) and Hodson and Beaumont (17); while Fox Wilson (9), in 1925, gives an account of the larvae severely damaging rhododendrons at Wisley. The species was also bred out from a collection of Tortrix larvae made on apple in the Wisbech district in 1925 (25), and was one of the species recorded by Miles (24) in 1929 in an experiment on Tortrix control on apple in the north-west. The larva has also been recorded by Speyer (34) as injurious to vines in greenhouses.

The species is widely distributed throughout Europe, being recorded from Astrachan (31), Belgium, on gooseberry (20), Constantinople (12), the Crimea, on fruit trees (27), France, on vegetable marrow (30), Norway (13), and from Stavropol (Russia) on blackcurrant, gooseberry, apple and elder (40). Barrett (3) also gives Northern Italy, Sardinia, Sweden, Dalmatia, Greece, Asia Minor and Eastern Siberia as areas where it occurs. It is also reported from Japan on

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[*] The chaetotaxy of this species and the comparison of its setal maps with those of other species will be described in a subsequent paper. This paper is the first of a series in which it is intended to include *Cacoecia lecheana* L., *Batodes angustiorana* Haw., *Tortrix diversana* Hb., *Argyroploce variegana* Hb., *Spilonota ocellana* Fabr., *Pandemis ribeana* Hb., *Pandemis heparana* Schiff., and *Peronea contaminana* Hb. Subsequent descriptions will be as comparative as possible, using the description of *C. podana,* as a basis of comparison.

citrus, boring into the fruit in the larval stages (5). It is not known to exist in America, but was intercepted once in U.S.A. during 1919-20 in a cargo of Rosa rugosa from Holland (32).

In recent years, the extent of the damage by Tortrix larvae to fruit trees in Great Britain has appeared to be on the increase, both as regards the spring damage to fruit buds and fruitlets and the autumn damage to fruit on the trees and in store. It was decided, therefore, to investigate the species responsible for this damage and a brief account of those bred out at East Malling in 1933 has already been given (15). Most of the work described below was carried out whilst both authors were working at East Malling Research Station, Kent, and was a development of work already initiated in the Entomological Section at East Malling.

II. DESCRIPTION OF STAGES AND BIOLOGY.

(a) The Egg and Egg Laying.

There does not appear to be any description of, or reference to, the egg of *Cacoecia podana* Scop. in the literature. The egg mass of a closely related species *Cacoecia (Archips) rosaceana* Harris, which is very similar in appearance, is, however, very well figured by Gilliatt (10).

The eggs of *C. podana* are laid in batches of about fifty, partly overlapping one another like the slates on a roof to form a compact egg mass (Plate I, Fig. 1). This egg mass is green in colour and closely resembles the upper surface of the leaf on which it is laid. When hatched, however, it is white in colour and much more easily seen. A very thin, waxy secretion is present over the whole of the outer surface of the unhatched egg mass. The individual eggs are green and wrapped in a very thin, almost transparent tissue. Each egg is roughly circular in shape with a diameter of about 0·68 mms. The average size of an egg mass is about 4 mms. by 2 mms., but there is great variation in size and shape owing to the variable number of eggs comprising each mass. The average number of eggs per mass is about fifty, but varies from six to one hundred and twenty. Observations made on moths in sleeves in the field showed one female to lay eight batches consisting of about three hundred and sixty-five eggs, another, seven batches of about three hundred and twenty-five eggs, and a third, seven batches containing about two hundred and sixty eggs.

In 1933 eggs were first noted in the field on June 12th and a few unhatched batches could still be found on July 25th, although by then many larvae from other batches were in the second instar. In 1934 eggs were first noted in the field on June 17th.

(b) Hatching.

The process of hatching is very similar to that obtaining in *Cacoecia crataegana* Hubn. (14), but owing to the more fragile nature of the egg mass emergence is much more rapid. Just prior to hatching the dark head of the larva can be seen clearly inside the egg, making the egg mass appear dotted with little black spots. In sheltered positions the white, hatched egg masses may remain on the leaves for some time, but otherwise are soon washed off by the rain.

(c) THE LARVA.

A very brief description of the larva is given by Stainton (35), Wilkinson (41), Morris (28), Barrett (3), Deakin (6) and Fox Wilson (9). These recorded descriptions all appear to refer to the mature larva or to the penultimate instar.

(1) FIRST INSTAR.

The first stage larva is 1·3 to 1·6 mms. long, with the abdominal prolegs bearing eight or nine crotchets in uniordinal series, and the width of the head capsule varying from 0·20 to 0·24 mms.

The head. The epicrania and frons are dark, smoky-brown, with the ocellar areas black, the epicranial sutures deep black and prominent and the adfrontal sutures indistinct. The antennae and maxillae are entirely pale. The following characteristics are the same throughout all the instars, clypeus pale, labrum red-brown, mandibles red-brown, darker at apex, labium with prementum as two dark brown lobes and a black anterior band.

The prothoracic plate is the same pale yellow colour as the rest of the body and this characteristic serves to distinguish this instar from all others.

The body. The general body colour is pale yellow above and below. The tubercules from which the setae arise are unicolorous with the rest of the body. In this and in all succeeding instars the setae are pale, except for a fine, dark, ring at the extreme base. The legs and anal plate are entirely pale yellow, the crotchets pale brown and the spiracles gray and indistinct.

Biology.

Immediately on hatching, the first stage larvae spin a fine web, usually alongside a leaf vein and begin to feed, almost always on the underside of the leaf. They generally remain in this position until after the first moult. In the field in 1933 first instar larvae were found on the leaves from June 26th to August 1st. In 1934 the first record of first stage larvae was made on June 22nd.

(2) SECOND INSTAR.

The second instar larva is 2 to 3·8 mms. long, with the abdominal prolegs bearing fourteen or fifteen crotchets in uniordinal series, and the width of the head capsule varying from 0·28 to 0·30 mms.

The head. The epicrania, frons, maxillae, and antennae are the same colours as in the preceding instar.

*The prothoracic plate** is now smoky-brown in colour with a very pale, almost transparent band along the anterior margin. This pale band is present in all succeeding instars, but sometimes, if the dark posterior margin of the head capsule is lying beneath it, then this pale band may be partially or totally obscured from view.

The body. This differs from the previous instar in that the crotchets of the prolegs are now golden brown in colour, the spiracles darker and more prominent, and gray to black irregular areas may appear on the thoracic legs.

Biology.

The second stage larvae generally leave the place where they fed in the previous instar and either web two leaves together and feed between them or else, on apple, web a leaf on to the surface of a fruit and feed on the skin and surface layers of the apple (Plate II, Fig. 1). On damson, pear and plum the majority of the larvae in all instars feed solely on the leaves, but some feed on the fruit, producing the same sort of damage to the skin as on apple (Plate II, Figs. 2, 3).

Larvae in the second instar were found in the field from July 4th to October 12th, 1933. Very occasionally larvae appear to over-winter in the second instar.

(3) THIRD INSTAR.

The third stage larva is 4 to 6 mms. in length, with the abdominal prolegs bearing sixteen to eighteen crotchets in uniordinal series and with the width of the head capsule varying from 0·35 to 0·47 mms.

The head. The epicrania and frons are dark brown to black, the former are usually black on the ventral margin. The epicranial sutures are black and the adfrontal sutures indistinct. The antennae have the first joint pale, except for a brown ring near the apex, and the second joint light brown, paler at base and apex. The first joint of the maxillae is pale, with a light brown band near the apex. The second and third joints are light brown. The maxillary lobes in this and in all succeeding instars are somewhat variable in colour, but are generally light to dark brown, with the apex pale. On the labium, the two setae arising near its base are surrounded by dark areas and the rest of the labium, except the prementum, is pale.

The prothoracic plate is now dark brown in colour with the usual pale anterior band. It is generally not as dark brown as are the epicrania.

The body. This is now slightly more yellow than in the two previous instars and the prothorax bears two faintly marked, smoky-brown, raised areas, ventral and anterior to the spiracle. The legs are extremely variable in colour, sometimes almost entirely pale, sometimes with dark areas, as in the next instar. The spiracles are black and distinct.

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* Frequently, when a larva in this and in all succeeding instars is fully fed and just about to moult, the thorax swells to such an extent that the prothoracic plate, which, being chitinous cannot swell, gets drawn out of position. This causes the production of a " yellow collar effect " between the prothoracic plate and the head. This, together with the view now afforded of the indented posterior margin of the head, gives the larva a very strange appearance so that it may easily be mistaken for another species.

Biology.

In the third instar, in autumn, the larvae tend to forsake the leaves and feed more on the fruit. Towards the end of October, or early November, depending on the season, they spin a dense silk cocoon beneath a bud scale, or in the crotch of a twig, or bind a dead leaf to a twig, and in this hibernaculum they spend the winter (Plate I, Figs. 2, 3). In late March or April, when the buds are bursting, they come out of hibernation and commence to feed, generally inside the fruit buds. Some larvae moult inside the hibernaculum either in winter or in early spring.

Sometimes in the autumn larvae get carried into the packing shed on the fruit: here they continue feeding on the skin of the fruit, especially on apples. Many of these larvae wander off, get lost in the packing shed and eventually die, either during the winter or else next spring, when they can find nothing to eat. Some of them get carried into the storage rooms and in ordinary stores (i.e. those without gas or low temperature) after feeding for a while they hibernate in the cracks in the boxes, or amongst the wrapping paper. Next spring they again feed upon the fruit (Plate II, Fig. 4), and may eventually complete their metamorphosis and give rise to adults. Others, which get carried into cold storage or into gas storage soon cease to feed and the majority of them die out over the winter; only very rarely do larvae appear to survive the winter under these conditions.

In the field in 1933, third instar larvae were found actively feeding from April 4th to April 29th and the next generation from August 8th to October 21st.

(4) FOURTH INSTAR.

The fourth stage larva is 6 to 8 mms. in length, with the abdominal prolegs bearing twenty-three to thirty crotchets in uniordinal series and the width of the head capsule varying from 0·53 to 0·68 mms.

The head. The entire head is the same colour as in the previous instar, except that the darker areas on the antennae, maxillae and labium may be almost black instead of brown.

The prothoracic plate is dark brown to black with the usual pale anterior band.

The body. The general body colour is yellow, with sometimes a slight green or gray tinge dorsally. The anal plate is generally yellow, but is occasionally more gray than the rest of the abdomen, though never really dark coloured or prominent. The two raised areas on the lateral margin of the prothorax are now almost black and more distinct than in the previous instar. The legs are very variable; the coxa and trochanter are mainly pale yellow, but with some smoky-brown areas. The femur, tibia and tarsus are generally smoky-brown, but pale at the base and apex. The tarsal claws are pale yellow to light brown in colour. Generally, the metathoracic legs are paler and the dark areas much less prominent than on those of the prothorax.

Biology.

The majority of the larvae over-winter in the third instar; a few, however, moult inside the hibernaculum so that in the spring the larvae as they commence feeding on the buds may be in either the third or the fourth instar. As the buds open, the larvae penetrate right inside, binding the parts together with silk and often severely damaging the blossoms so that they cannot function.

In 1933 fourth instar larvae were found in the field from April 4th to May 4th and in 1934 from April 6th to April 29th.

(5) INSTAR FIVE.

The fifth stage larva is 8 to 12 mms. in length, with the abdominal prolegs bearing thirty-one to thirty-seven crotchets in an irregular biordinal series and the width of the head capsule varying from 0·72 to 1·14 mms.

The head. The head capsule is the same colour as in the previous instar, except that in some cases the adfrontal sutures are now pale and distinct.

The prothoracic plate is the same in colour as in instar four.

The body. The general body colour is usually similar to that of the previous instar, but in some specimens the dorsal and lateral margins of the body are dirty yellow-gray, or dirty gray-green and the ventral surface dirty yellow. The two raised areas on the prothorax near the spiracle are black and more prominent, and the dark areas on the legs are generally more pronounced than in instar four.

Biology.

By the time the larvae are in the fifth instar, apples are generally in bloom and on some varieties the fruitlets are formed. The larvae now forsake the buds and bind two leaves together, feeding between them. Sometimes, they merely shelter between these leaves and come out to feed on the blossoms or young fruits. On apples, they may bite pieces right out of the young fruitlets, or so damage them that a corky scar or pimple develops over the wound (Plate I, Fig. 4). This type of damage, but caused by a different species of Tortrix larva, is figured also by Gilliatt (10, 11). On damsons, pears and plums the damage seems to be confined much more to the buds, blossoms and leaves.

Fifth instar larvae were found in the field, in 1933, from April 20th to May 12th and in 1934, from April 22nd to May 10th.

(6) Sixth Instar.

The sixth stage larva is 12 to 16 mms. in length, with the abdominal prolegs bearing forty-one to forty-eight crotchets in a biordinal series and the width of the head capsule varying from 1·25 to 1·50 mms.

The head. The epicrania are now chestnut brown to pale chestnut in colour, with the ventral surfaces dark brown and the ocellar area black. The frons is usually darker than the epicrania. The epicranial sutures are black and prominent and the adfrontal sutures pale and distinct. The rest of the head capsule is the same colour as in instar five, except that the dark areas round the two setae, near the base of the labium, are larger and more pronounced.

The prothoracic plate is dark brown and rarely black, with the usual pale anterior margin.

The body. All the same colour characteristics of the body in this instar are the same as in the mature larva.

Biology.

The habits of the sixth instar larvae are similar to those of the fifth, except that they tend to feed more on the leaves and less on the blossoms and fruitlets. They do not usually roll one leaf over onto itself, but generally bind two separate leaves together, or a dead leaf onto a green leaf, or a green leaf onto a twig, and use this merely as a shelter, moving out to feed elsewhere.

Pupation sometimes, but not often, occurs in this instar.

In the field, sixth instar larvae were found from May 1st to May 20th, 1933, and May 3rd to May 19th, 1934.

(7) Seventh Instar.

The mature larva is 14 to 16 mms. in length when newly moulted, and 20 to 24 mms. long when fully grown. The abdominal prolegs bear fifty-two to fifty-seven crotchets in biordinal series and the width of the head capsule varies from 1·56 to 2·0 mms.

The head. The epicrania are light chestnut brown in colour, sometimes somewhat darker ventrally, and with the ocellar area black. The frons is the same colour as the epicrania, never darker. The epicranial sutures are black, very prominent, and the adfrontal sutures pale, distinct. The clypeus is pale, the labrum red-brown and the mandibles red-brown, but darker at apex. The first joint of the antennae is pale, except for a brown ring near the apex and the second joint is brown to almost black, except for a pale area at base and apex. On the maxillae, the first joint is pale, with a brown band near the apex and the second and third joints are light to dark brown. The maxillary lobes are light to dark brown in colour, but pale at the apex. On the labium the prementum is present as two dark brown lobes with a black anterior band.

Near the base of the labium, surrounding the two big setae which arise there, are two dark chitinous areas. The rest of the labium, except the prementum, is pale.

The prothoracic plate has a broad, pale, semi-transparent band along the anterior margin. Along the posterior and posterior-lateral margins there is an irregularly defined very dark brown or black area. In the centre, the rest of the plate, a roughly semi-circular area, is light brown, much paler and less red-brown in colour than are the epicrania.

The body. The general body colour is extremely variable. It may be dark green, or yellow-green, or pale gray-green, or light grass-green, or dirty yellow-gray, or even blue-gray. It is generally slightly darker dorsally than ventrally, whatever the colour may be. Sometimes this distinction in colour between the two surfaces is very marked ; in some specimens the dorsal surface is dark green and the ventral surface pale grass-green. The dorsal surface of the body is covered with tubercules, from which the setae arise ; these tubercules are generally paler than the rest of the body. The dark rings at the base of the pale setae are often surrounded by gray irregular areas, slightly darker than the pale tubercules, near the apex of which they are situated.

The anal plate is a dirty gray colour, or else unicolorous with the rest of the abdomen. Sometimes, however, the body is a dirty gray-green colour and the anal plate a pale yellow-green with darker spots.

The legs are also very variable in colour ; the coxa and trochanter are mainly unicolorous with the body, but with some dark areas. The femur, tibia, and tarsus are generally dark brown to black, except the base and apex, which are usually pale. The tarsal claws are light brown in colour. Generally, the metathoracic legs are paler and the dark areas less prominent than those of the prothorax. The spiracles are dark brown to black, and prominent. The prothorax bears two prominent, raised, black, chitinous areas just ventral and anterior to the spiracle.

Biology.

The habits of the seventh instar larvae are similar to those of the sixth. When fully fed they pupate between the leaves, generally in their last shelter place.

In 1933, seventh instar larvae were found in the field from May 13th to June 3rd, and in 1934, from May 17th to June 9th.

(d) THE LARVA IN RELATION TO DYAR'S HYPOTHESIS.

The cast larval head capsules were measured unmounted and, in addition, measurements were made of the unmounted head capsules of larvae pickled in Pampel's fluid. It was found that measurements made of the same head capsule when on the living larva, or when cast, or when pickled, gave almost identically the same result. The following table shows that within limits, Dyar's Hypothesis holds true for the larvae of *C. podana.*

It is suggested also that the number of crotchets on the abdominal prolegs is a valuable guide to the instars of the larvae which should not be overlooked.

TABLE I.

Head measurements of larvae of C. podana *in successive instars.*

Instar.	Measurements of larval heads in mms.			Calculated widths in mms.
	Number.	Range.	Average.	
1	31	0·20—0·24	0·22	0·19
2	37	0·28—0·30	0·28	0·28
3	65	0·35—0·47	0·41	0·41
4	42	0·53—0·68	0·60	0·60
5	64	0·72—1·14	0·90	0·88
6	29	1·25—1·50	1·39	1·28
7	29	1·56—2·00	1·77	1·87

The individual records from which these figures were taken have been deposited with the Natural History Museum at South Kensington, London.

In this table, the ratio as calculated from the second, third and fourth instars is 1·46.

No observations were made as to what extent the variation in the size of the head capsules, especially in the last two instars, is a sexual factor.

(e) THE PUPA.

A brief description of the pupa has been given by Wilkinson (41) and by Barrett (3).

The following description refers solely to the empty pupal cases and is thus limited to abdominal characters (Text-Fig. 1). According to Mosher (29),

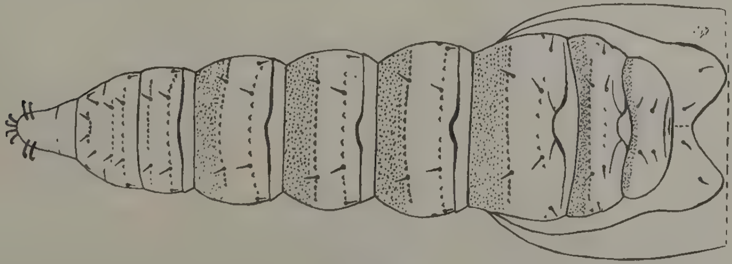

FIG. 1.
Ventral view of empty pupal case of *C. podana.*

however, these are the most useful characters for separating the species of a family.

The colour varies independently of sex, from light brown to almost black. The transverse conjunctiva is somewhat lighter coloured and uniformly reticulated without prominent brown spots. The total length in the male varies from 8·5 to 12·5 mms., with an average of 11 mms., and in the female from 11 to 14 mms., with an average of 12·4 mms.

The setae are present and arranged as on the mature larvae.

The sexual openings are arranged as in other Tortricid pupae, the female having two distinct openings which, however, often appear confluent. The anus is situated at the caudal end of the tenth segment, on an anal rise, which does not bear setae. The cremaster is slightly longer than broad, somewhat flattened dorso-ventrally and bears two pairs of hooks arising in a dorso-lateral position; also two pairs of terminal hooks. The proleg scars are not distinct.

Spines are present on the dorsum of abdominal segments 2 to 9 inclusive and are arranged as follows: second abdominal, a few small spines in an indistinct row anteriorly and a distinct posterior row. Third abdominal, two rows, the anterior row shorter than the posterior. Fourth to eighth abdominal inclusive, two rows of approximately equal length. Ninth abdominal, a few scattered spines representing a posterior row. In general, the anterior rows on all segments are composed of fewer and larger spines than the posterior rows.

On abdominal segment 1, there is dorsally a narrow transverse depression running across the anterior margin of the segment. The anterior margins of segments 2 and 3 are curled over on the mid-dorsal line, forming an arch over a transverse depression, which lies in the same position as on segment 1, but is much more marked. On segments 4 to 7 inclusive the anterior part of the segment is marked off dorsally by a transverse ridge. This ridge has a mid-dorsal, caudally directed bend.

Biology.

Pupation may occur in the sixth as well as in the seventh instar. When fully fed the larvae pupate between the leaves, generally in their last shelter place. The empty pupal cases often project some distance from the leaves which formerly protected them.

In the field in 1933, pupae were found from May 19th to June 19th, and in 1934, from May 23rd to June 26th.

(f) THE MOTH.

The moth is described by Wilkinson (41) and by Barrett (3), so that no description is attempted here. Fox Wilson (9) says of it: "When at rest this species resembles a dead leaf."

Biology.

The habits of the moth are also briefly described by Barrett (3) and several writers record it as on the wing in June and July. In 1924 Theobald (39) took adults in a light trap from June 25th to August 31st.

In the field, in 1933, moths were taken by beating the trees from June 9th to July 27th. In 1934 the first moth was taken on June 11th. In sleeves in the field copulation was observed within two days of emergence and egg laying took place within four days of copulation.

III. ECONOMIC IMPORTANCE.

(a) Host Plants and Distribution.

The recorded food plants of the larva in Britain are: apple, apricot, azalea, blackberry, blackthorn, hawthorn, hop, ivy, oak, pear, plum, raspberry, rhododendron, rose and vine. The larva has also been recorded feeding on "Big Bud" on black currants. In addition, one of the writers and A. M. Massee bred out the species at East Malling from larvae found on cherry, damson, quince and walnut (15). The writers have also bred out the moth from larvae obtained on beech in Gloucestershire.

The moth is recorded by several authorities as common and widely distributed throughout Britain. The writers have found it exceedingly common throughout Kent and have also bred it from larvae obtained from Berks., Bucks., Cambs., Cheshire, Devon, Essex, Glos., Hereford., Herts., Lancs., Lincs., Norfolk, Somerset, Suffolk, Sussex, Worcester., and Wisbech and Isle of Ely.

Theobald (37) has recorded that in 1923 at one plantation of 150 acres near Newington of mixed apples, pears and plums, there was such a severe infestation of Tortrix as to strip all the leaves off some of the trees and cause damage estimated at £2,300. The species present were *Tortrix heparana,* 50 per cent.; *Tortrix ribeana,* 25 per cent.; *Antithesia pruniana,* 20 per cent.; and the remainder *T. podana, T. rosana* and *Spilonota roborana.* In other orchards at Ash and Sandwich and in other parts he recorded *T. podana,* as often reaching 50 per cent. and sometimes *T. ribeana,* as much as 60 per cent. of the species present. Generally, he recorded *heparana, ribeana* and *podana,* as the commonest species.

Hey and Massee (15) in 1933 found that in every case of Tortrix damage that they investigated the most important species was *C. (T.) podana.* From a total of 3,350 larvae collected in the packing shed of a cold store in September, 85 per cent. were *C. podana,* 10 per cent. *Batodes angustiorana* and 5 per cent. *Spilonota ocellana.* These larvae were responsible for damage to as much as 34 per cent. of the fruit. So that in Kent at least, *C. podana* is a pest of no little economic importance.

In 1934 one of the present writers found the larvae of *C. podana* exceedingly common in commercial apple orchards and cider orchards in Devon, Glos. and Somerset. The only other species that was at all common there in June was *Argyroploce variegana* Hb. In several orchards in Herts., Sussex and Worcs., in May and June 1934, *C. podana* and *A. variegana* were the commonest species and occurred in the proportion of about 2 to 1, together comprising over 95 per cent. of the total Tortrix population on apple trees at that time.

(b) INJURY TO PLANTS.

Fox Wilson (9) records the larvae severely injuring rhododendron at Wisley in 1925 and gives a photograph of the damage done to the leaves. He records that on rhododendrons, contrary to their usual custom of fastening two leaves together by a silken web and feeding between them, the larvae fed openly and did not attempt to conceal themselves between the leaves.

Speyer (34) has recorded the larvae as injurious to vines in greenhouses.

On fruit trees the larvae are one of the species responsible for the well-known Tortrix damage to buds, flowers and young fruits. They feed inside the opening buds, leaves and blossoms, frequently webbing them together and thus spoil the proper development of the foliage and fruit. By their feeding on the young fruits they may damage them so severely that they fail to develop, or else, principally on apple, cause the formation of corky scars or pimples (Plate I, Fig. 4), which, when the fruit comes to be marketed, severely detracts from its value.

This spring damage has long been recognized, but more important than this is a type of damage in autumn to the surface of the fruit of apples, pears, plums (Plate II, Figs. 1, 2, 3), and sometimes damsons. This autumn damage, especially on apple, has increased enormously of recent years and now, in some areas, is so serious as to cause the species to assume the status of a major pest.

Prior to the work of Hey and Massee (15) at East Malling in 1933, this species had not previously been recorded as responsible for damage to the fruit in the autumn. In 1917, however, *Batodes angustiorana* Haw., a species often found associated with *C. podana*, was recorded damaging apples in store at Mill Hill (4); it was not noted again until it was recorded in the Eastern Province in 1929 (26). Since then, damage to apple fruits in store has been recorded several times in the monthly reports of the Ministry of Agriculture, and *Batodes angustiorana* has generally been thought to be the culprit, but it is highly probable that most of the damage was really due to *C. podana*. In

1931, however, there is a substantial record for *B. angustiorana,* for a moth was bred out by Adkin (2) from a larva feeding on a wrapped apple in store in March. In 1932 Massee (21) described and figured damage to apple fruits in store at East Malling, giving a brief description of the larva responsible and a short account of the life history of the species which he recorded as *Batodes angustiorana* Haw. (?). From subsequent observations on the prevalence of the different species by Hey and Massee in 1933 (15), there is little doubt that this record refers not to *B. angustiorana,* but to *C. podana.* The description reads: "The larva is of a light yellowish-green colour, with the head and second segment black"; this applies to *C. podana,* but not to *B. angustiorana,* for this latter species, in all instars, has the head and a major portion of the prothoracic plate a pale wax, or a very light brown colour.

The importance of this autumn damage, which may continue in the packing shed and in storage, has already been emphasized by Massee (21) in 1932, and by Hey and Massee (15, 16) in 1933, so that no further emphasis is necessary.

On cherry, quince, walnut, raspberry, blackberry and hawthorn, no damage was ever observed on the fruits either in spring or autumn. In fact, except on hawthorn, the species was not found very commonly on these hosts. On hawthorn, however, it appears to be quite common in several counties, the larva webbing the leaves together in big bunches and feeding in the centre of the webbing. On walnut it was observed only at the Research Station, where it fed on the new shoots as they developed. From raspberry and blackberry, the larvae were brought in to the writers by Mr. W. Steer, where they were webbing the leaves together after the manner of the Raspberry Shoot Moth, *Aspis udmanniana,* from which species, however, they are very easily distinguished. On cherry, the larvae were found in several localities in Kent; they always appeared to be in association with larvae of *Cacoecia lecheana* L. The larvae were obtained on pear, plum and damson from several counties, but they were not as common or as injurious on these hosts as on apple, the damage being confined mainly to the foliage and blossoms.

IV. CONTROL.

In 1928, Miles (24), using several proprietary brands of tar-distillate washes at 7½ to 8 per cent. concentration, obtained what he terms "a very satisfactory control" of larvae of *Cacoecia rosana* L., *Cacoecia podana* Scop. and *Argyroploce ochroleucana* Hb.; "all of which," he says, "appear to spend the winter as young larvae about the trees."

In 1929, Kent (19), referring to over-wintering Tortricid larvae, says, "They enter the bursting buds before there is a reasonable chance of coating foliage or shoot with lead arsenate, and I find a 10 per cent. tar-distillate wash the best means of dealing with them."

The general consensus of opinion among growers in 1933, whom the writers questioned on the matter, was, that most certainly arsenate of lead in the spring did not effectively control the pest, even when applied over a period of years. This was borne out by observations made on the Research Station, where the pest was extremely prevalent on certain of the plots, in spite of the fact that lead had been incorporated in the pre-blossom and some of the post-blossom sprays on these plots and the surrounding ones for a period of years. This failure to obtain control of the larvae on fruit trees with lead arsenate appears to be due to the fact that the larvae feed so much inside buds and blossoms and between leaves webbed together; that is, always in places where a good coating of lead cannot be deposited. That the larvae on other hosts can be controlled by lead arsenate has been shown by Fox Wilson (9), who says that on rhododendron, where the larvae feed openly, a good control can be obtained by the use of a lead arsenate and Saponin wet spray, or a lead arsenate dust. Similarly, Speyer (33), advocates the use of repeated applications of a spray of lead arsenate and Saponin for the control of the larvae on vines under glass.

xxi 56.

As regards the control obtained with tar-distillates used at 10 per cent., the same growers were of the opinion that a better control was obtained at this strength than at lower concentrations, but that the control obtained was not good enough to compensate for the extra cost of the higher concentration.

Actually, in 1933, no attempt was made by the writers to devise special means of control, but observations were made on the effect of ordinary routine spraying on the larvae. Unfortunately, counts made of the percentage of fruits damaged in the autumn are no criterion of the value of winter or spring washes, because a generation of moths occurs in between and causes re-infection by egg-laying over a wide area. In order, therefore, to obtain some data on the value of tar-distillates used at 10 per cent., as compared with the same material at 5 per cent., counts were made of the number of dead and living larvae on prunings from the two differently treated plots. Unfortunately no prunings were available from trees entirely unsprayed with tar-distillate, so that no "control" figures were obtained. On the 10 per cent. plot, from a total of just over two hundred larvae examined, the number dead in their cocoons was 64 per cent.; while on the 5 per cent. plot, from one hundred and thirty-five larvae, 38 per cent. were dead. These figures, however, are too small to draw definite conclusions.

Another means of Tortrix control is by the use of light traps. Theobald (39) gives a list of the species of economic importance that he caught and states: "The traps undoubtedly failed as a means of catching male Winter moths, for in no case were they taken in any large numbers. The tables, however, show that large numbers of Tortrices are eliminated, and as these are not completely dealt with by spraying, the use of these light traps may probably prove of great benefit in districts where these pests are serious, and they are certainly worth an extended trial by growers who suffer from Tortrix damage. They, at the same time, attract moths from the hedgerows, from which source Tortrix infection may often come.''

Speyer (33), however, after trying light traps in glasshouses, concluded that they could not be recommended under glass.

Hey and Massee (16) have shown that, when present on fruit placed in gas storage, the larvae can be controlled by the use of certain gas mixtures.

V. PARASITES.

The prevalence of parasitism in the larva seemed to be very low as compared with a closely related species, *C. lecheana* L. In *podana*, from a total of nearly four hundred larvae collected in the field, only 4 per cent. were parasitized, whereas in *lecheana*, from a total of thirty larvae as many as 25 per cent. were parasitized.

No egg or pupal parasites of *podana* were observed; but altogether one Tachinid and seven Hymenopterous species were bred from the larvae.

From one larva which had over-wintered in a store and was discovered feeding on an apple in May, inside the wrapping paper, numerous larvae of the gregarious Braconid *Macrocentrus abdominalis* F. emerged. These quickly pupated and in three weeks gave rise to eighteen females and five males. It seems likely that the parasites must have been present in the larva throughout the winter. Several more specimens of *Macrocentrus abdominalis* F. were bred out during the summer, also some of the solitary species, *Macrocentrus thoracicus* Nees. Three specimens of a third species of *Macrocentrus* were also bred out; this was a solitary species and not as common as the other two. It has not yet been possible to determine the exact species, but it appears to be one new to this country.

The sub-family Ophioninae (Ichneumonidae) was represented by several females of *Omorga* (?) *biloba* Thoms. and one female of *Pristomerus vulnerator* Panz. The only other Hymenopterous parasites obtained were several specimens of *Apanteles longicaudis* Wesm. and a few of an undetermined species of *Apanteles*.

The only Dipterous parasite bred out was a single specimen of *Nemorilla notabilis* Mg.

VI. SUMMARY AND ACKNOWLEDGMENTS.

An account has been given of the life history and habits of the moth, *Cacoecia podana* Scop.

The egg and egg laying have been described and the morphology (excluding the chaetotaxy) and biology of the larva and pupa have been studied.

The measurement of the larval head capsule in relation to Dyar's Hypothesis has been discussed.

The damage done by the larva to fruit trees and other host plants, and to apple fruits in store, has been described.

The effect on the larva of routine control measures has been observed.

The parasitism of the moth has been studied; one Dipterous and seven Hymenopterous species of parasites have been bred out.

The writers wish to acknowledge their indebtedness to all those who so very kindly sent in larvae, or allowed collections to be made in their orchards or fruit stores. They also wish to thank Messrs. G. J. Kerrich, G. E. Nixon, J. F. Perkins and Dr. A. Roman for the identification of the Hymenopterous parasites, Mr. J. E. Collin for identifying the Dipterous parasite and Mr. T. Bainbrigge Fletcher for his identification of the moths.

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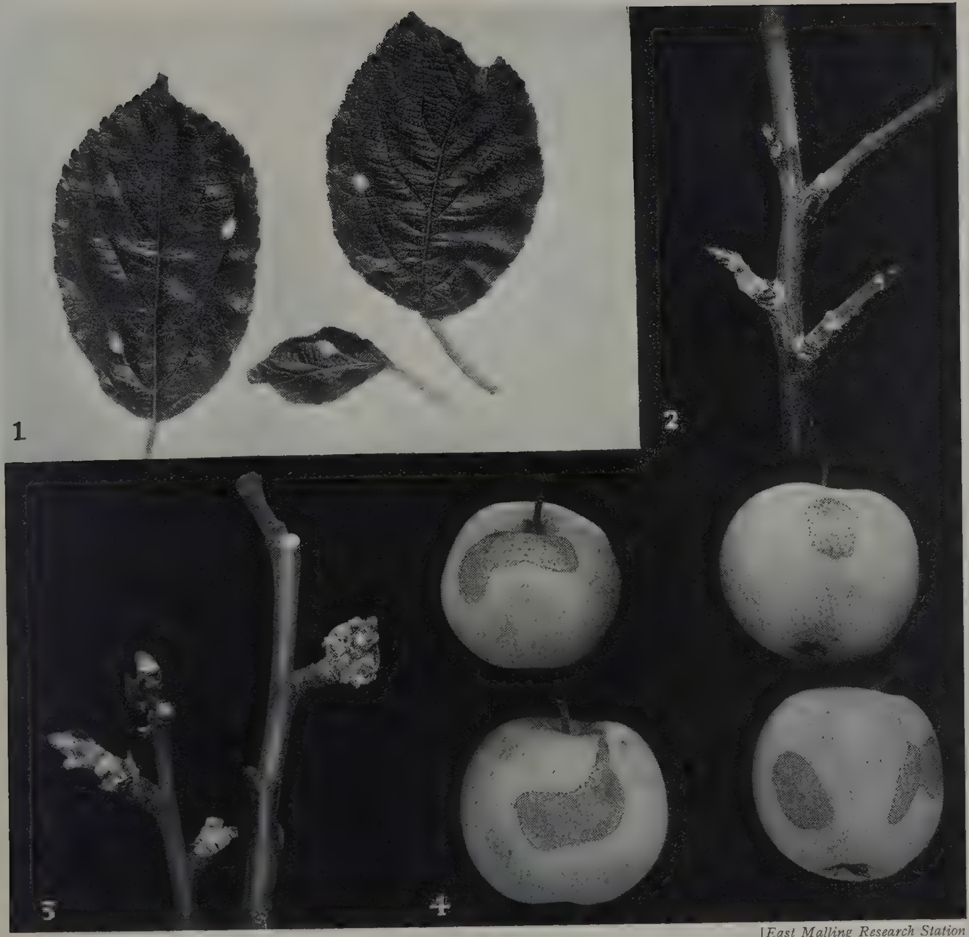
(*Received* 29/6/34.)

PLATE I.



[East Malling Research Station

Fig. 1. Hatched egg-masses of *C. podana* on apple leaves.

Fig. 2 and Fig. 3. Hibernation sites of larvae of *C. podana* on apple twigs.

Fig. 4. Scars produced on apple fruits by the spring feeding of larvae of *C. podana*.

PLATE II.

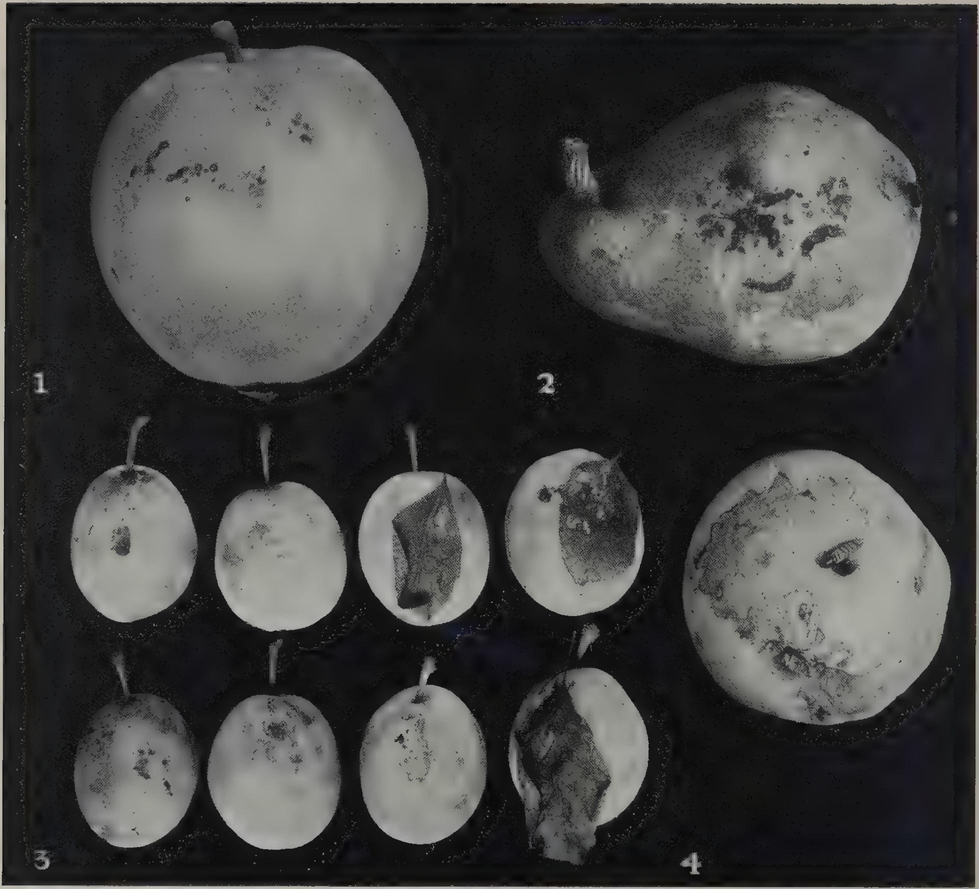


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Fig. 1. Autumn damage to surface of apple fruit by larvae of *C. podana*.

Fig. 2. The same type of damage on pear.

Fig. 3. The same type of damage on plum, also showing the webbing of the leaf to the fruit.

Fig. 4. Pupa of *C. podana*, and damage by the larvae feeding on apple fruit, in storage at ordinary temperature, from October to April.

IODIZED WRAPS FOR THE PREVENTION OF ROTTING OF FRUIT

By R. G. TOMKINS

Low Temperature Research Station, Cambridge

SERIOUS losses are continually occurring in the storage and transport of food through the growth of moulds and fungi. Practical methods for reducing these losses are urgently required.

Fungal rotting of fruit can be checked either by preventing infection by mould spores or, if this is not possible, by storing under conditions which retard their development.

It is obviously impossible to avoid some infection of fruit during the time it is growing, and rots can often be traced to orchard infections. Infection may also take place between the time of picking and packing, for example, the infection of oranges and apples by spores of *Penicillium*, and care and speed of handling at this stage may greatly reduce wastage at a later date. But as all fruit, however carefully grown or handled, is to some extent infected with fungae spores, it is generally necessary to take special measures if the development of rots is to be avoided.

Dipping the fruit in certain disinfecting solutions has been tried with some measure of success—for example, a borax dipping treatment is often used for the prevention of green rot of citrus. The addition to the air of small amounts of such substances as acetaldehyde, ammonia and sulphur dioxide have all been used at times to reduce the extent of wastage of fruit. But none of these methods are easily or widely applicable on a commercial scale, either because they add too much to labour costs of handling, or because the maintenance of the required conditions present serious engineering difficulties. In fact, when due consideration is paid to the various economic factors and to the difficulties encountered in the various stages of handling, there appears to be one method which would be generally useful for the control of rotting, and that is the use of especially treated wraps.

Treated wraps are already widely used to overcome certain troubles. Wraps impregnated with mineral oil are used to prevent scald of apples (1). Wraps impregnated with copper sulphate are used to prevent the rotting of pears (2). These wraps do not, however, reduce the incidence of the more usual types of rotting. Copper sulphate contained in the wraps can only prevent the spread of rots from one fruit to another, and cannot prevent moulds growing on the surface of the fruit. If growth on the surface of the fruit is to be prevented, the wrapper must be impregnated with a substance which can diffuse from the paper, reach the surface of the fruit and there inhibit mould growth.

For substances to be suitable for this purpose they must fulfil certain requirements, viz. :—

(1) They must be volatile so that they diffuse from the paper, but only slightly volatile so that they diffuse away slowly.

(2) When present in small quantities they must prevent the growth of moulds on fruit, and this they must do without causing damage to the fruit.

(3) They must not hasten the ripening of the fruit or dissolve in fruit juices to give a characteristic taste.

(4) They must not render the fruit toxic to human beings.

It is easy to find substances which satisfy some of these requirements, but difficult to find substances which satisfy all of them. There is, however, one substance at least which may do so. That substance is iodine.

Many fruits, though not all, when wrapped in paper impregnated with small quantities of iodine remain free from rots or fungal growth for much longer periods than fruits wrapped in untreated paper, and do so, moreover, without suffering any damage, without their ripening processes being appreciably advanced and without their flavour being impaired. Eggs, also, when stored in flats and fillers treated with iodine remain free from moulds though stored in a saturated atmosphere.

The effectiveness of iodine in these respects is due to its particular physical, chemical and biological properties.

THE PHYSICAL AND CHEMICAL PROPERTIES OF IODINE.

Iodine is a solid which at ordinary temperatures has a small but appreciable vapour pressure. Thus, if impregnated into a fruit wrap, it slowly diffuses from the wrap and reaches the surface of the fruit. The rate at which it does this depends largely on the temperature.

Iodine combines with starch to form a loose compound. It similarly combines with cellulose, the material from which paper is made. It also combines with potassium iodide.

These properties are made use of in preparing wraps. The cellulose nature of the tissue paper reduces the vapour pressure of the added iodine and retards the rate at which it diffuses away. The vapour pressure can also be reduced by adding potassium iodide. The length of time during which free iodine remains in the wraps can be modified by the amounts of iodine and potassium iodide added.

THE FUNGICIDAL PROPERTIES OF IODINE.

The effect of iodine on the rate of growth of the fungus *Trichoderma lignorum* (and there is every reason to believe that other fungi are similarly affected) has been investigated in some detail by a method especially evolved for the study of the effect of certain chemical substances on the growth of fungi.

The fungus was allowed to grow in a liquid culture medium contained in a Petri dish in such a way that growth starting in the centre spread out regularly in every direction to form a circular mat. It was possible to add iodine to the liquid medium before germination had begun and so find its effect on germination, or it was possible to add it after growth had started and so find its effect on growth. The medium used was a 2 per cent. solution of malt. It was buffered by adding:

either 1 per cent. potassium hydrogen phthalate which gave a pH of 4;
or 1 per cent. potassium dihydrogen phosphate which gave a pH of 5;
or 1 per cent. sodium acetate which gave a pH of 6;
or 1 per cent. mixture of potassium dihydrogen and potassium phosphate which gave a pH of 7.

The exact relations between concentration of iodine and retardation of growth rate need not be considered here. The following are some of the more important features of the effect of iodine on the germination and growth of moulds.

(1) The presence of iodine retards the germination of spores,
    (*a*) by prolonging the latent period of germination, i.e., the time for the germ tubes to appear.
    (*b*) By reducing the rate of elongation of the germ tubes.

(2) The concentration of iodine necessary to inhibit germination depends on the acidity of the medium in which germination takes place. The more acid the medium the less iodine is required. The amounts required are given in Table I. The concentrations given are the maximum

TABLE I.

Effect of Iodine on Germination and Growth of Trichoderma lignorum.

pH of culture medium.	4	5	6	7
Concentration of iodine necessary to prevent germination	N/10,000	N/4,000	N/500	N/500
Concentration of iodine necessary to reduce the rate of growth to half its normal value	N/10,000	N/2,500	N/600	N/300
Concentration of iodine necessary to prevent further growth	N/500	N/500	N/200	N/100

Note: N Solution = 1·27% iodine.

amounts which have been observed in any experiment to be necessary to check germination. Smaller concentrations than those recorded may be sufficient to retard germination, for it has frequently been observed in an experiment set up in triplicate in which spores are allowed to germinate in the presence of 0, 1, 2, 3, etc. parts of iodine, that though there is in general a uniformity of behaviour, germination may often fail in one or two or even all of the three comparable sets at even relatively low concentrations. This lack of uniformity cannot at present be accounted for.

(3) If iodine is added to a solution in which a colony is growing, the rate of growth is reduced to a lower value. In the presence of a definite concentration of iodine the growth rate, as measured by the rate of radial spread, remains constant as the colony increases in size.

(4) The concentration of iodine necessary to reduce the rate of growth depends, as with germination, on the pH of the medium. The concentrations necessary to retard and inhibit the further growth of an established colony are, however, considerably greater than those required to retard or inhibit germination of spores or the growth of a very small colony.

(5) The first addition of iodine appreciably reduces the growth rate. Further additions do not have so marked an effect. Thus the concentration necessary to inhibit growth is more than twice the amount necessary to reduce the growth rate to half its normal value. Table I records the concentrations necessary both to reduce the growth rate to half its normal value and to inhibit growth.

The fact that iodine delays and retards germination in smaller quantities than are required to delay growth of older mycelia, that it is more active in an acid medium, and that the first additions are more effective than later additions, explains in large measure why iodine can be present in sufficiently small quantities to prevent the germination of spores or the continued activity of newly established colonies and yet not damage the fruit tissue on which growth of moulds would normally occur.

The Preparation of Wraps.

Wraps have been prepared by allowing ordinary wraps to absorb a known volume of iodine solution and then to dry at ordinary temperatures.

Generally the solution of iodine used has consisted of 12·7 gm. iodine, 10 gm. of potassium iodide, 200 cc. of water and 800 cc. of rectified spirit. The high proportion of rectified spirit assists in securing rapid and even penetration and rapidity of drying. Sheets of tissue paper approximately 50 cm. by 75 cm.

readily absorb 15 cc. of the solution. In making wraps in quantity it is convenient to take five sheets of tissue paper, to place them on a sheet of glass and pour on 75 cc. of the solution. Uniform absorption is got by wearing a rubber glove and smoothing the wet paper. The sheets are allowed to dry and each is then divided into six smaller sheets 25 cm. square. Each sheet then contain approximately 30 milligrams of free iodine.

THE USE OF IODIZED WRAPS IN THE STORAGE OF FRUIT.

ORANGES.

Oranges were wounded by brushing with a hard scrubbing brush, inoculated with spores of *P. digitatum*, wrapped in plain wraps or wraps impregnated with iodine or iodoform and stored in ventilated tin boxes at definite temperatures. Table II summarizes the result of one such experiment. Iodine wraps reduced the extent of mould attack without damaging the fruit. Wraps impregnated with iodoform also reduced the extent of rotting. These cannot, however, be used, for the iodoform taints the fruit.

TABLE II.

Percentage Number of Oranges Sound after Storage for Definite Times.

Temperature.	Type of wrap.	6	9	12	15	19	28 days
25°	Plain	20	—	10	10	10	5
	Iodine	80	—	65	65	60	55
	Iodoform ..	80	—	75	70	60	60
18°	Plain	40	15	10	10	5	5
	Iodine	70	50	40	40	40	35
	Iodoform ..	55	40	40	40	35	25
10°	Plain	—	55	25	15	5	5
	Iodine	—	100	75	45	35	30
	Iodoform ..	—	75	45	30	15	5
5°	Plain	—	—	—	—	55	10
	Iodine	—	—	—	—	70	35
	Iodoform ..	—	—	—	—	50	10

In order to find the most suitable amounts of iodine and potassium iodide to be included in the papers, comparable samples of wounded and inoculated oranges were stored wrapped in papers impregnated with various amounts of iodine and potassium iodide.

Table III records the results and shows that 25 cm. square wraps which have been treated with 1 per cent. potassium iodide and 1—2 per cent. of iodine give on the whole the most satisfactory results. Such wraps contain approximately 25—50 milligrams of free iodine.

TABLE III.

Effect of amounts of Iodine and Potassium Iodide contained in the Wrap on the extent of Rotting.

Strength of solution absorbed by the wrap.	% sound after 5 days at 18° C.	% sound after 10 days at 10° C.	% sound after 26 days at 5° C.
Plain wraps	15	30	5
½% iodine + 0% potassium iodide ..	20	25	10
,, + 1% ,, ..	35	30	25
,, + 2% ,, ..	15	35	55
1% iodine + 0% potassium iodide ..	45	55	20
,, + 1% ,, ..	25	50	50
,, + 2% ,, ..	40	70	45
2% iodine + 0% potassium iodide ..	35	40	70
,, + 1% ,, ..	30	70	65
,, + 2% ,, ..	45	55	65

GRAPES.

Grapes are particularly liable to mould attack and the most striking results obtained with iodized paper have been in controlling mould growth on grapes.

Table IV records the results of some storage experiments. Retardation of mould development over a wide range of temperature is observed and at intermediate temperatures the storage life is prolonged by some two to three times.

TABLE IV.

The storage life of Grapes in Plain and Iodized Wraps.

Temperature of storage.	Type of wrap.	Days in storage before appearance of moulds.		
		Blue Grapes (Belgium).	Blue Grapes (Belgium).	Green Grapes (Spain).
42°	Plain	—	4	3
	Iodine	—	>6	6
32°	Plain	—	5	4
	Iodine	—	15	14
25°	Plain	—	5	4
	Iodine	—	11	15
18°	Plain	5	5	8
	Iodine	12	19	30
10°	Plain	10	11	30
	Iodine	25	28	40
5°	Plain	—	19	>40
	Iodine	—	40	>40

TOMATOES.

Tomatoes in storage are liable to extensive mould attack.

Green tomatoes wrapped, some in plain and some in iodized wraps were stored at various temperatures. The colour changes proceeded normally from green to red at 20° and 15° C. At 10° and 5° C. the colour changed from green to yellow. The colour change was in no instance influenced by the wrap. Attack by mould was reduced at all temperatures by iodized wraps (*cf.* Table V.)

TABLE V.

The storage of Tomatoes in Plain and Iodized Wraps.

Total in Samples of ten showing Rots after certain times in Storage.

Temperature.	Type of wrap.	17	28	38	44	54 days.
20° C.	Plain ..	2	10	—	—	—
	Iodine ..	—	2	2	3	5
15° C.	Plain ..	—	3	5	6	8
	Iodine ..	—	0	0	1	2
10° C.	Plain ..	—	4	5	8	9
	Iodine ..	—	0	0	0	0
5° C.	Plain ..	—	2	5	5	10
	Iodine ..	—	0	0	0	0

It is obviously laborious to wrap each fruit. However, iodized paper shavings or iodized cork dust might be used.

Table VI shows the result of storing tomatoes in iodized shavings. Iodized shavings appreciably reduce the extent of rotting. But the effect on appearance is more striking than the recorded figures suggest. Tomatoes stored in the ordinary way produce luxurious growths of mould from the calices and are frequently covered by a superficial growth of *Rhizopus* spreading from one rotten fruit.

TABLE VI.

The percentage number of Tomatoes suffering from Rots when stored with and without Iodized Shavings.

	% Nos. rotting after 20 days.			% Nos. rotting after 30 days.	
	Ordinary Temperature.	25°	18°	10°	5°
Ordinary storage	42	45	43	89	79
Storage in iodized shavings ..	19·5	30·2	16	51	49

Tomatoes stored in iodized shavings remain free from this superficial growth and the calices remain fresh and green.

PLUMS.

If unripe plums are wrapped in iodized paper they ripen abnormally. The green colour persists for longer periods than normal and browning of the skin may follow. Ripe plums, when similarly treated, may suffer no damage. Plums have been stored in layers between iodized paper. Reduction in the amount of rotting has always been obtained by storing with iodized paper, sometimes with little scalding, sometimes with severe scalding.

Thus the use of iodized shavings for the control of rotting of plums may for this reason be useful for certain varieties only, and for plums gathered in a fully ripe condition.

APPLES.

Certain varieties of imported apples have been wounded and inoculated with spores of *P. expansum* and then stored, some in iodized wraps, some in plain wraps.

Though rotting by *P. expansum* has been largely controlled by iodized wraps, the apples have sometimes shown a marked browning of the surface and sometimes a more rapid rate of ripening. Not all varieties suffer in this way, but the fact that some varieties are damaged indicates the need for further experiment before the method is used on a large scale.

PEACHES.

Peaches are similar to plums in their behaviour to iodine.

One variety wrapped in iodized wraps ripened normally and remained free from brown rot, while another variety ripened abnormally.

OTHER FRUIT.

Passion fruit wrapped in iodized wraps remained free from mould while similar fruit in plain wraps became mouldy.

Melons wrapped in iodized wraps have been stored without suffering damage.

THE EFFECT OF IODINE ON THE RIPENING PROCESSES.

In storing fruit it is necessary not only to reduce mould growth but also to control the incidence of physiological diseases and the course of ripening. The ideal wrap would be one which effectively retarded ripening and also controlled physiological and fungal diseases. Certainly no suggestions for the treatment of wraps should be made without considering the physiological effects of such treatment.

Some fruits give off ethylene, or a substance which has all the properties of ethylene, at a certain stage of ripeness. This can be demonstrated by leading a current of air over the fruit and then over germinating peas. In a current of ordinary air peas germinate normally, whereas in a current of air taken over ripe apples, or in a current of air to which a small amount of ethylene has been added, they germinate abnormally. As ethylene hastens ripening, fruits stored in bulk tend to ripen more uniformly and more rapidly than fruit stored singly. If ethylene could be absorbed as it is evolved, this bulk effect would be eliminated. Bromine absorbs ethylene and it was thought that iodine in the wrappers might similarly absorb ethylene. Unfortunately this is not the case, for in air taken over apples wrapped in iodized wraps the germination of peas was inhibited. Therefore retardation of ripening cannot be achieved in this way. Iodine contained in wraps does, however, often destroy the smell of fruit which usually pervades the storage atmosphere, without affecting the smell or taste of the fruit itself.

The effect of iodized wraps on the rate of respiration of tomatoes and grapes has been measured.

The respiration of unwrapped tomatoes was first followed. Half the number were then wrapped in iodized wraps and the others in plain wraps and measurements of the respiration continued. The rate of respiration of green tomatoes increases as the colour changes from green to red and then decreases, and so it is not possible to give any one figure for the rate of respiration. No appreciable increase or decrease in the rate of respiration nor in the way the rate changed in time was observed in fruits wrapped in iodized wraps as compared with fruit in plain wraps. This is in agreement with the fact already mentioned that the ripening of tomatoes as judged by colour change was unaffected by iodine contained in the wraps.

The respiration of small bunches of green Almeria grapes was also measured. It was found to be constant over a period of ten days. The average rate of respiration in cc. per Kg. of fresh weight per hour at 18° C. were :

for 3 bunches wrapped in plain paper ..	46·75	45·7	50·3
for 3 bunches wrapped in iodized paper ..	46·30	45·7	53·3

The respiration is seen to be affected to but little extent by iodine in the wraps.

The respiration of blue grapes was also measured. The rate of respiration decreased continuously during the experiment and therefore no single value can be given. In this instance, however, the respiration was increased by about 20 per cent. by wrapping in iodized wraps.

As, however, mouldiness rather than over-ripening determines the storage life of grapes, the increase in respiration in some cases does not seem likely to form a serious objection to the use of iodized wraps.

SUMMARY.

The fungal rotting of fruit in storage can be considerably reduced by wrapping or packing the fruit in paper impregnated with suitable amounts of iodine.

Grapes and tomatoes, when wrapped in paper impregnated with small amounts of iodine, are kept free from rots or fungal growth for longer periods than when wrapped in untreated paper, without their appearance or flavour being in any way impaired.

Tests on a larger scale are planned to confirm and extend the results obtained in the laboratory. Investigations are in progress to ascertain the degree to which iodine is absorbed by fruit from iodized wraps. The question of compliance with the Public Health (Preservatives, etc., in Food) Regulations will require consideration at that stage.

The rotting of apples, plums and peaches has also been reduced by storage in iodized wraps. The appearance and the ripening of some varieties of these fruits are, however, adversely affected by this treatment.

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CHROMOSOME NUMBER AND POLLEN GERMINATION IN PEARS

By A. A. MOFFETT
John Innes Horticultural Institution, Merton

In recent years cytological studies have thrown considerable light upon the genetical behaviour and causes of sterility in cultivated fruits. Thus pollen abortion or irregularity has in many cases been shown to be due to chromosome irregularities at the reduction division during pollen formation. Many of our fruits, instead of possessing only two homologous sets of chromosomes, are polyploids with three or more chromosome sets. Polyploids with an even number of chromosome sets, such as tetraploids with four, hexaploids with six, etc., are invariably more fertile than those having an odd number of sets, such as triploids with three and pentaploids with five. In the odd numbered polyploids the chromosomes cannot divide in equal numbers at the reduction division in germ-cell formation, and consequently irregularities occur, followed by a high degree of pollen sterility. Cytologically, cultivated apples may be divided into two distinct groups—diploids having thirty-four chromosomes and triploids with fifty-one chromosomes. Pollen germination tests have shown that these two classes show a considerable difference in pollen fertility. The triploids always have a low proportion of good pollen, less than 30 per cent. germinating in sugar solutions. In the diploids on the other hand the pollen germination is much higher, varying from 50 to nearly 100 per cent. Thus the diploid with the poorest pollen has a considerably higher pollen germination than the best triploid.

A study of a number of varieties of pears was undertaken to determine to what extent triploidy occurred among cultivated varieties, and if there was a sharp differentiation into two classes according to pollen infertility, as in apples. Pollen mother cell material was fixed in all varieties examined. In diploids it was found easy to make an accurate count at metaphase of meiosis, but in the triploids irregularities of division made it much more difficult to obtain the correct number. Good somatic divisions were obtained however in developing petals of most varieties, and it was thus possible to make accurate counts of the somatic chromosome number. The chromosome behaviour at meiosis in diploid and triploid varieties has been described in a previous paper (Moffett, 1934).

Most of the material for cytology and pollen germination tests was obtained from trees growing at the John Innes Horticultural Institution. Material of other varieties was kindly provided by the Director of the East Malling Research Station.

TABLE I.

	Chromosome number.			Percentage pollen germination.						
	Kobel.	Miedzyrzechi.	Moffett.	Kobel.	Miedzyrzechi.	Moffett.	Florin.	Passecker.	Ziegler and Branscheidt.	Maximum germination.
André Desportes	34	—	—	54	—	—	30-70	—	—	54
Baronne de Mello	—	—	34	—	—	—	—	—	—	—
Belle des Abres	—	34	—	—	66	—	—	—	—	66
Beurre Bosc	—	—	34	—	—	59	—	77	77	77
Beurre Clairgeau	—	—	34	—	—	81	30-70	69	85	85
Beurre Dumont	—	34	—	—	36	—	—	—	—	36
Beurre de Mortillet	—	—	34	—	—	—	—	—	—	—
Beurre de Naghin	—	34	—	—	43	—	—	—	—	43
Beurre Fougeray	—	—	34	—	—	72	—	—	—	72
Beurre Giffard	—	34	34	—	—	21	30-70	—	—	30-70
Beurre Hardy	34	—	34	72	—	82	30-70	—	76	82
Beurre Six	—	—	34	—	—	61	—	—	—	61
Beurre Superfin	—	—	34	—	—	—	70-100	—	20	>70
Bon Chrétien d'Hiver	–	34	—	—	—	—	—	—	—	—
Charles Marchal	—	34	—	—	—	—	—	—	—	—
Chalk	—	—	34	—	—	78	—	—	—	78
Clapp's Favourite	—	34	—	—	55	—	30-70	38	63	63
Comtesse de Paris	—	—	34	—	—	60	—	—	—	60
Conference	—	—	34	—	—	72	0-30	—	—	72
Dr. Jules Guyot	—	34	34	—	54	42	—	—	11	54
Doyenne du Comice	34	—	34	78	—	74	30-70	87	86	87
Durondeau	—	—	34	—	—	50	—	—	—	50
Fertility	—	—	34	—	—	69	—	—	—	69
Fondante Thirriot	34	—	—	31	—	—	30-70	—	—	30-70
Frühe von Trevoux	34	—	—	29	—	—	—	—	—	29
Gansel Bergamot	—	—	34	—	—	—	70-100	—	—	>70
Glou Morceau	34	—	34	31	—	59	0-30	15	—	59
Herzogin Elsa	—	—	34	—	—	33	70-100	78	—	78
Le Brun	34	—	—	43	—	—	—	—	—	43
Louise Bonne de Jersey	34	—	—	54	—	—	30-70	59	82	82
Marie Louise	—	—	34	—	—	57	—	—	—	57
Marguerite Marillat	—	—	34	—	—	3	0-30	—	—	3
Napoleon	—	—	34	—	—	—	70-100	—	24	>70
Neue Poiteau	34	—	—	31	—	—	30-70	—	18	30-70
Olivier de Serres	—	34	—	—	40	—	0-30	—	31	40
Orange Bergamot	—	—	34	—	—	—	—	—	—	—
Passe Crassane	—	34	34	—	56	26	0-30	—	—	56
St. Luke	—	—	34	—	—	58	—	—	—	58
Souvenir du Congrés	—	—	34	—	—	46	0-30	—	25	46
Triomphe de Vienne	—	34	—	—	60	—	—	3	55	60
Williams' Bon Chrétien	34	—	34	46	—	56	30-70	55.	73	73
Barikerbirne	51	—	—	11	—	—	—	—	—	11
Beurre d'Amanlis	ca46	—	51	25	—	7	0-30	—	6	25
Beurre Diel	ca45	51	51	6	5	12	0-30	22	23	23
Catillac	—	—	51	—	—	18	—	—	—	18
Conseiller de la Cour	44-48	—	51	13	—	—	—	—	—	13
Doyenne Bussoch	—	—	51	—	—	—	—	—	—	—
Pitmaston Duchess	—	—	51	—	—	10	0-30	—	1	10
Rateau Gris	—	51	—	—	9	—	—	—	—	9
Schweizer Wasserbirne	47	—	—	13	—	—	—	—	7	13
Theilersbirne	48	—	—	22	—	—	0-30	—	—	22
Uvedale's St. Germain	—	—	51	—	—	9	—	—	—	9
Vicar of Winkfield	55	—	—	4	—	—	0-30	24	13	24

CHROMOSOME NUMBERS.

Thirty-four varieties were examined and of these twenty-seven were diploids and seven triploids. Previous workers have all reported triploid varieties amongst the material examined. Kobel (1926) found ten out of seventeen varieties to be diploids. The remaining seven he reports as having aneuploid chromosome numbers with 2n ranging from 45 to 55. Three of these varieties have since been examined and found to be triploids. As no other aneuploid forms have been reported, it is probable that all the varieties reported by Kobel to have intermediate numbers will prove to be triploids. A list of the varieties of pears studied by different workers, and their chromosome numbers, is given in Table I.

Natividade (1932) reports twelve diploids and three triploids amongst Portuguese varieties. These varieties are not widely cultivated and have not been included in the Table.

Of those so far examined about 22 per cent. of the pear varieties and 21 per cent. of the apple varieties are triploids. The agreement in the proportions is therefore very close.

POLLEN GERMINATION.

Pollen germination tests were carried out in hanging drop cultures using cane sugar solutions. The pollen of some varieties was germinated on agar and sugar, but this method gave no better results, and it was found more difficult to make counts. Preliminary tests were made in 5 and 10 per cent. sugar solutions, but the pollen of some varieties formed very short tubes, which burst almost immediately, making accurate counting impossible. It was decided therefore to test the pollen germination of each variety in several concentrations of sugar. Six concentrations were used: 5, 10, 15, etc. up to 30 per cent. The varieties examined, the chromosome number and the percentage of pollen germinating in sugar solutions of different concentrations are given in Table II.

Pollen germination tests of pear varieties have been carried out by many workers previously. [Kobel (1927), Florin (1927), Passecker (1930), Ziegler and Branscheidt (1927), Miedzyrzechi (1933).] All the above give the actual percentage of pollen germinated, except Florin, who divides his results into three classes, (1) average pollen germination not above 30 per cent., (2) average pollen germination 30-70 per cent., (3) average pollen germination at least 70 per cent. Florin used a 10 per cent. sugar solution, Passecker used solutions of 5 to 20 per cent. going up by 5 per cent.; Ziegler and Branscheidt used 2½, 10, 15 and 20 per cent. sugar.

The highest pollen germination obtained by the investigators in any sugar solution has been tabulated in Table I.

TABLE II.

Pollen Germination in %.

Variety.	Chromosome number (2n).	Concentration of Sugar Solution.						Maximum germination.
		5%	10%	15%	20%	25%	30%	
Beurre Hardy ..	34	79	82	79	20	59	50	82
Beurre Clairgeau ..	34	*b	b	38	30	37	81	81
Chalk	34	46	65	69	78	67	47	78
Doyenne du Comice ..	34	b	b	49	42	65	74	74
Conference	34	19	18	72	60	50	37	72
Beurre Fougeray ..	34	44	70	72	65	63	17	72
Hessle	—	19	35	63	55	71	46	71
Fertility	34	7	26	69	25	30	30	69
Beurre Six	34	18	45	42	61	—	—	61
Princess	—	—	—	—	50	54	60	60
Beurre Bosc	34	10	60	28	54	40	37	60
Comtesse de Paris ..	34	14	26	40	51	60	60	60
Glou Morceau ..	34	b	b	38	32	48	59	59
St. Luke	34	14	28	46	55	58	37	58
Marie Louise	34	48	49	53	57	43	43	57
Williams' Bon Chrétien	34	42	50	56	36	34	4	56
Durondeau	34	15	28	31	50	31	—	50
Souvenir du Congrés	34	38	46	42	39	27	24	46
Dr. Jules Guyot ..	34	b	36	20	12	42	18	42
Charles Ernest ..	—	21	32	34	35	30	30	35
Hersogin Elsa ..	34	28	33	13	28	12	23	33
Passe Crassane ..	34	9	—	15	26	17	—	26
Beurre Giffard ..	34	12	21	20	12	13	11	21
Alexander Lucas ..	—	2	3	4	3	6	6	6
Marguerite Marillat ..	34	0	—	3	1	—	—	3
Catillac	51	3	3	18	8	—	5	18
Beurre Diel	51	12	9	11	9	4	6	12
Pitmaston Duchess ..	51	0	10	—	9	3	2	10
Uvedale's St. Germain	51	1	9	9	9	9	—	9
Beurre d'Amanlis ..	51	2	4	4	7	1	0	7

* b=pollen burst.

The results of pollen germination in pear varieties show considerable divergences. Not only do the results of different investigators differ widely for the same variety, but it is found that a repetition of the germination test, using pollen from the same tree in the same sugar solution, may give different results. Passecker, and Ziegler and Branscheidt, tested pollen of varieties obtained from different sources or from the same source in different years and found that the percentage of pollen which germinated varied greatly. Thus the varieties Conference, Glou Morceau, Olivier de Serres, Passe Crassane and Souvenir du Congrés, reported by Florin to have pollen germination between 0 and 30 per cent., have been found to show a considerably higher germination percentage by other authors. It is possible that in some of these cases the low percentage of germination may be due to 10 per cent. sugar not being the optimum concentration for these varieties. On the other hand I obtained low results with the varieties Beurre Giffard and Herzogin Elsa, but Florin found a considerably higher germination. Sometimes also a sample of pollen will give unexpectedly poor results in a particular sugar solution, e.g. Beurre Hardy in 20 per cent. sugar (see Table II.)

Marguerite Marillat is exceptional amongst the diploid varieties. This pear has been tested in two consecutive years in many different concentrations of sugar, and in every case the germination has been very low or nil. The pollen of this variety is very irregular, and only a very few full sized grains occur, the majority being shrivelled and clumped together. Florin also reports this variety to have a low pollen germination (0—30 per cent.).

Although the diploid varieties show much variation in their pollen germination, they have on the whole (with the exception of Marguerite Marillat) a considerably higher percentage germination than the triploids, which have been found by all investigators to have a low germination. Morphologically also the pollen of the triploids differs from that of the diploids. Many of the diploid varieties which have a low pollen germination have a high percentage of pollen which appears morphologically good. In the triploids on the other hand the pollen grains are very irregular in size, a large percentage being shrivelled or micro-grains, while many of the larger grains possess no pore. The pollen tubes formed in triploids are usually short and thick and seldom grow as long as the tubes in diploid varieties.

An examination of the Tables shows, therefore, that while the difference is not so distinct as in apples, cultivated pears fall into two groups according to their chromosome numbers. The diploid pears are apparently more sensitive to environmental conditions affecting pollen growth than are the diploid apples.

It is evident from the differences in germination of pollen from the same tree in different years that environmental factors such as temperature, moisture, etc. must affect the pollen germination considerably.

Since odd multiple polyploids are as a rule relatively infertile, it would be expected that the triploid varieties of pears would not be sufficiently productive to be widely cultivated. In pears as in apples, however, a very low proportion of fruit to flowers is sufficient to give an economic yield, and often the formation of one or two seeds is sufficient for the development of fruit. From the point of view of pollenizers however the diploid varieties, with their more abundant pollen and higher percentage germination, would be more effective in the field than the triploids.

This investigation has been carried out in conjunction with Mr. M. B. Crane. Other aspects of the problem of sterility and incompatibility in pears are under investigation at this Institution.

SUMMARY.

1. The chromosome numbers in thirty-four varieties of pear have been determined. Twenty-seven were diploids and seven triploids. Approximately 22 per cent. of the varieties examined by various workers have been found to be triploids.

2. The diploid varieties on the whole have a higher percentage of pollen germination than the triploids. The distinction between the two classes is not so clear-cut however as in apples. The optimum concentration of sugar for germination varies with the variety, and varies in the same variety from year to year.

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BOOK REVIEWS

SPECIFICATIONS AND METHODS OF ANALYSIS FOR CERTAIN INSECTICIDES AND FUNGICIDES. Bulletin No. 82, Minstry of Agriculture and Fisheries, London, 1934. Price 3d. (Post extra.)

This bulletin gives specifications of ten standard products used as insecticides or fungicides, or in the case of soft soap, for spraying purposes. The list is substantially the same as that published in Advisory Leaflet No. 9 of the Ministry of Agriculture, the only additional specifications being for Bordeaux Powder, Burgundy Powder and Cheshunt Compound. The specifications in the present bulletin are, however, fuller and embody information which was not available when the previous list was prepared in 1921.

In the second part of the bulletin are given the prescribed methods of analysis for the products specified. This is an important advance because the analysis of some of the insecticides and fungicides is somewhat complicated and unless the methods are standardized the results may not express correctly those constituents upon which the value of the spray depends.

It will be noted that no mention is made of other important spray materials such as the tar-distillate and oil washes, pyrethrum, derris, nor of the new " spreaders ". This is because the information at present available is not sufficient to allow of specifications or methods of analysis being standardized, but the increased interest which is being taken in spray products, both by research workers and manufacturers, ought to lead within a reasonable time to some of these blanks being filled.

The standards given in the bulletin have no legal force but the specifications are expressed more effectively (and with " shall " instead of " should ") than in the previous bulletin. They have, however, the official sanction of the Ministry of Agriculture, the Government chemist and the National Farmers' Union. The Members of the Association of British Insecticide Manufacturers and certain other firms have also agreed to conform to the standards.

Now that standardization has been introduced it is to be hoped that the further important step of providing means for the testing of spray products will follow. With the aid of standard preparations it is possible in many cases to exercise effective control of disease. There are, however, proprietary preparations, and until some authoritative body is in a position to test them both the user and the maker are at a disadvantage. The former fearing to be imposed upon may refuse to consider any but standard non-proprietary articles, whilst the latter may be checked in his initiative, with the result that valuable materials are lost for spraying purposes.

There are probably numerous chemicals used industrially which if tested would be found to possess properties which would make them most serviceable in connection with spraying. Two examples of such application come to mind—" Shirlan " and " Lorol ", both of which are products largely used in the textile industry, whilst the tar-distillate washes arose from the use of creosote as a wood preservative.

W.G.

DICTIONARY OF TERMS RELATING TO AGRICULTURE, HORTICULTURE, FORESTRY, CATTLE BREEDING, DAIRY INDUSTRY AND APICULTURE IN ENGLISH, FRENCH, GERMAN AND DUTCH. By T. J. Bezemer. George Allen & Unwin, Ltd., London, 1934, 8vo, pp. 248, 25s.

The only real test of such a dictionary is its constant use. Since, however, the very recent publication of this work precludes such a test, recourse must be had to quicker though less reliable methods of evaluation. Is it comprehensive and is it reasonably accurate, and how does it compare with other dictionaries in this respect ? As regards comprehensiveness the editor admits in his preface that he has been forced to make selection of the words to be used. Thus he states that not all injurious and useful insects are included, but only those which are most important to agriculture. The difficulty of deciding border-line cases is obviously immense and results of such decision cannot satisfy everyone. Moreover, he points out, being the first dictionary in the field to deal specifically with agriculture, it obviously cannot make any claim to completeness, and suggestions are invited as to sins of omission and commission. Further the work does not pretend to be an encyclopaedia. It is not a defining or a descriptive dictionary and, as is noted by the editor, the reader is assumed to be aware of the meanings of words in his own language. That there are indeed somewhat serious omissions soon becomes apparent. May we win the editor's gratitude by suggesting a few ? Abscission, crab apple and crab stock, banana and tropical horticultural crops in general, capsid, carbolineum and winter wash, citrus, cover crop, dewberry, die-back, ditcher, dusting in sense of dry spraying, fig, fireblight, free stock, hedger, iodine, labourer, layout, leaf scorch, loganberry, mulch and sod mulch, pecan, persimmon, pistache, ploughman, plot, pyrethrum, rootstock, sport, smudging, stoolbed, trowel, vernalization, viticulture. Here it should be noted that in one case at least the foreign equivalent is given but with a curious English translation. Thus the foreign equivalent of rootstock appears as unterlage, sujet and onderlaag, but the English version is given as parent stock, wildling and wild stock, which is an inaccurate and inadequate translation. Next may we suggest that the following terms are perhaps somewhat precious ? Though understandable by the aid of mother wit or of the Oxford dictionary they leave us irritated even if enlightened : basic slacks, gladiole, inflorescense, ligh-trequirement, nursery man, assart, assayer, period for wich . . . ., sabulous, agricultural university, infectuous abortion, parturation, polnish wheat, viniculture.

The editor asks for comments. May we take this to mean that he contemplates a second edition at no distant date ? The idea is excellent. We believe that horticulturists from all parts of the globe are just ready for such a dictionary, provided it is comprehensive and accurate. Let them be approached forthwith to remedy sins of omission and commission. And now in our enthusiasm broaching the subject of a second edition may we point to the serious research work being carried out in Italy and her colonies and in Spain and Spanish speaking countries of South America and urge the inclusion of these two further languages. Lastly, we insist that poetic licences are not granted to lexicographers and that in a dictionary of this nature, to be used by English and American horticulturists, plain English is preferable to rarities and solecisms? To ensure their elimination the manuscript of the revised version should before going to press be submitted to the close scrutiny of an Englishman of some agricultural knowledge and general education.

What then are the merits of the first edition of this dictionary, with which, after all, we are here concerned. It is well printed, of small size (approx. 6″ × 8¼″ × 2″) and weighs about 2¾ lb. It thus compares very favourably with any one dictionary from German, French or Dutch giving even approximately the same information. It contains, moreover, a large number of expressions which will not be found in many much larger, ordinary foreign dictionaries :—green manuring, damping off, bitter pit, the different types of grafting such as cleft, crown, root, top, whip graft, etc., bordeaux mixture, glassiness in apple, silver leaf may serve as examples. Such expressions appear in the English section with their foreign equivalents and in their proper place in the foreign sections, and their inclusion is most valuable.

Taken then in its entirety this work, though not the very perfect horticultural dictionary for which we have sighed so long, is definitely the nearest approach as yet to that ideal. As such, despite its shortcomings, it can be warmly recommended to the studious horticulturist.

D.A.

Reviews of the following books received will appear shortly :

THE GENETICS OF GARDEN PLANTS. By M. B. Crane and W. J. C. Lawrence.

STATISTICAL METHODS FOR RESEARCH WORKERS. By R. A. Fisher, Sc.D., F.R.S. Fifth Edition, revised and enlarged.

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